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Title of Thesis: Regulation of Pituitary Beta-Endorphin Release;
Role of Serotonin Neurons

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ABSTRACT

Title of Dissertation: Regulation of Pituitary β-Endorphin Release:
                          Role of Serotonin Neurons

Diana Sapun Malcolm, Doctor of Philosophy, 1983

Dissertation directed by: Dr. Gregory P. Mueller, Associate Professor,
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β-Endorphin-like immunoreactivity (β-END-LI) is present in high-
est concentration in the anterior (AL) and intermediate lobe (IL) of the
pituitary gland from where it is released in response to pain and other
stimuli. Several lines of evidence suggest that serotonin neurons influ-
ence nociception via mechanisms which involve opiate receptors and
the release of endogenous opiate peptides within the central nervous sys-
tem (CNS) and possibly from the pituitary. The purpose of the present
research was to determine whether serotonin neurons are involved
in the regulation of pituitary β-END-LI secretion and therefore provide
evidence for a functional relationship between serotonergic-stimulated
release of pituitary β-END-LI and endogenous mechanisms of analgesia.

Since the pituitary is the major source for blood-borne β-END-LI,
the effects of serotonergic drugs on plasma levels of β-END-LI was used
to study serotonergic control over pituitary β-endorphin. Plasma
β-END-LI levels were measured by a RIA which can detect <10 pg β-END$_{1-31}$
standard and which recognizes β-END's precursor, β-lipotropin (β-LPH)
and the shorter and acetylated forms of β-END$_{1-31}$ on an equimolar basis.
The administration of serotonin precursors, L-tryptophan (TRYP) and
5-hydroxytryptophan (5-HTP) elevated plasma levels of β-END-LI in a

manner which paralleled changes in brain serotonin content. Quipazine, a drug which mimics the action of serotonin at its post-synaptic receptor, elevated plasma β-END-LI levels in a time and dose-related manner. Furthermore, the rise in plasma levels of β-END-LI in response to quipazine treatment was completely blocked by cinanserin, a serotonin receptor antagonist. Fluoxetine, a drug which inhibits serotonin reuptake and therefore prolongs serotonin's activity within the synapse, elevated basal levels of plasma β-END-LI from $0.32 \pm .04$ ng/ml to 1.61 ± 0.14 ng/ml by 15 min post-injection and this rise was significantly attenuated by cyproheptadine, a serotonin receptor blocker. Central administration of serotonin (5 µg, 15 min) into pentabarbital-anesthetized rats significantly elevated plasma β-END-LI over control values. Pargylene, a drug which prolong's serotonin's action at the synapse by inhibiting enzymatic degradation of serotonin, also elevated circulating levels of β-END-LI in a time and dose-related manner. Taken together, these results suggest that serotonin neurons exert a stimulatory influence over the secretion of pituitary β-endorphin.

Immobilization stress evoked a 12-fold rise in plasma β-END-LI as compared to control values. 5,7-Dihydroxytryptamine (5,7-DHT), a serotonin neurotoxin, decreased hypothalamic serotonin content by 77% and significantly attenuated the rise in plasma levels of β-END-LI released in response to stress. Ether exposure (90 sec) increased basal levels of plasma β-END-LI 3-fold and potentiation of serotonergic activity by fluoxetine pretreatment further elevated this rise in plasma levels of β-END-LI. Together these results suggest that a serotonergic mechanism is involved in the stress-induced release of pituitary β-endorphin.

Cultured AL and IL cells secrete distinctive forms of β-END-LI which reflect the differential processing of the β-END precursor between the two lobes. The AL releases β-LPH and $β-END_{1-31}$, the only β-END peptide with analgesic properties. In contrast, the IL releases the shorter and acetylated forms of β-END which are not analgesic. Since β-LPH secretion clearly distinguishes β-END-LI release by the AL from the IL, changes in plasma levels of β-LPH-sized immuno-reactivity [resolved by gel filtration chromatography (Sephadex G-50,)] were interpreted to reflect β-END-LI release from the AL in vivo. TRYP, 5-HTP, quipazine and fluoxetine treatments all increased plasma β-END-LI resembling β-LPH and β-END in molecular size suggesting serotonergic stimulation of $β-END_{1-31}$ from the AL. Furthermore, dexa-methasone, a drug which selectively inhibits AL β-END-LI release, lowered circulating levels of β-LPH and β-END immunoreactivity in control and fluoxetine-treated animals. Serotonin did not exert a di-rect effect on β-END-LI release by cultured AL (or IL) cells indicating that its effects in vivo are mediated via actions on the CNS which re-sult in the release of hypothalamic β-END releasing factor(s).

In summary, these findings suggest that serotonin neurons stimu-late the in vivo release of β-END-LI from AL corticotrophs and thus evoke the release of opiate-active $β-END_{1-31}$ and β-LPH from AL corticotrophs. This mechanism appears to mediate, in part, the basal and stress secretion of β-END peptides into blood. Present in vitro studies indicate that serotonin does not influence β-END-LI secretion by a direct action on the pituitary gland but rather causes the release of a hypothalamic β-END releasing factor, most likely, corticotropin-releasing factor.

REGULATION OF PITUITARY β-ENDORPHIN RELEASE:

ROLE OF SEROTONIN NEURONS

by

Diana Sapun Malcolm

Dissertation submitted to the Faculty of the Department of Physiology
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DEDICATION

I dedicate this dissertation to my parents, Gregory and Irene Sapun for their loving support and to my husband Jim and son Jimmy for their love and understanding.

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I wish to thank Dr. Gregory Mueller for his constant guidance, advice and support throughout my research studies and during the preparation of this dissertation. Dr. Mueller has sought to create an atmosphere of scientific inquiry and enthusiasm within the laboratory for which I admire him. He has also set a fine example for good scientific research for which I respect him. I am most grateful for Dr. Mueller's genuine friendship anad his patience and encouragement during my development from a graduate student to a doctoral research scientist.

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INTRODUCTION

Historical Perspective

One of the oldest areas of biological research is the study
of morphine, the major alkaloid in opium poppy extracts, which
produces profound effects on the central nervous system (CNS)
resulting in a large variety of responses, the most notable of
which are analgesia, euphoria, and addiction. The discovery of
opiate receptors in the CNS of animals (Simon et al., 1973; Pert
& Synder, 1973; Terenius, 1973) and man (Hiller et al., 1973)
raised the question why receptors for alkaloids present only in
plants should exist in the nervous system of mammals. A reasonable
explanation seemed to be that opiate receptors and their natural
ligands served a physiological role within the mammalian CNS, and
thus the search for endogenous ligands for the opiate receptor
began.

Earlier evidence from neurophysiological studies had suggested
the existence of endogenous opiate substances. In 1969, Reynolds
observed that electrical stimulation of the periaqueductal gray
region of the rat produced analgesia. These studies were followed
up and expanded on by Liebeskind, Mayer, Akil and their co-workers
(Mayer et al., 1971; Akil et al., 1976) who also made the vital
discovery that naloxone, a specific opiate antagonist, partially
reversed stimulation produced analgesia. Furthermore, it was shown
by Mayer and Hayes (1975) that electrically-induced analgesia exhibits
cross-tolerance with morphine-induced analgesia. These results were

best explained on the basis of the existence of an endogenous opioid substance that could be released by electrical stimulation.

The discovery of an endogenous factor in extracts of pig brain which had opiate-like properties in in vitro bioassay systems (the mouse vas deferens and the guinea pig ileum) was first reported by John Hughes in 1975. Terenius and Wahlstrom (1975) independently isolated a water-soluble material from rat and calf brain which was characterized by its ability to compete with labeled opiates for receptor binding. These two materials appeared to be very similar to an opioid material extracted from calf brain by Pasternak, Goodman and Snyder (1975). All of these endogenous opioid substances were water-soluble, heat-stable, degradable by peptidases, and had a molecular weight of 800-1200.

Subsequently, the active factor present in extracts of pig brain was purified and characterized by Hughes et al. (1975) and found to consist of two pentapeptides. The pentapeptides were named enkephalins and were identical in sequence except for one amino acid. Hence, the two peptides were distinguished on the basis of this amino acid difference and accordingly called methionine-enkephalin and leucine-enkephalin. Both peptides were found to resemble morphine in their actions on the guinea pig ileum and mouse vas deferens bioassays. Interestingly, methionine-enkephalin (met-enkephalin) was shown to be about 20 times more potent than morphine in the bioassay, and leucine enkephalin (leu-enkephalin) was somewhat less potent than the methionine pentapeptide.

At the time of the publication of the structure of met- and leu-enkephalin, Hughes et al. (1975) were aware of the occurence of met-enkephalin in the carboxy-terminal position (positions 61-65) of the

pituitary hormone, β-lipotropin (β-LPH), isolated from sheep by C.H. Li in 1964. This remarkable finding gave rise to the isolation of a number of other peptides from hypothalamus and pituitary gland extracts which possessed opioid properties and shared a common sequence with β -LPH. The proliferation of endogenous opiate-like peptides prompted Dr. Eric Simon to suggest the name endorphin (for endogenous morphine-like material) for this family of peptides.

It was during the isolation of β-LPH from camel pituitary glands, that Dr. Li and Mr. Chung (1976a) obtained a 31 amino acid peptide that had an amino acid sequence identical to the carboxyterminal 31 residues of ovine β-LPH (β-LPH$_{61-91}$). Most important this peptide was found to be the most potent of the endogenous opioid peptides isolated thus far (Loh et al., 1976). It was named β-endorphin by C.H. Li. Within a few months of its initial discovery, several researchers (Bradbury et al., 1976; Cox et al., 1975; Loh et al., 1976; Rubinstein et al., 1977) independently isolated β-endorphin from brain extracts and demonstrated that this peptide was biologically active in a wide variety of opiate test systems both in vitro and in vivo. In 1976, two laboratories (Chretien et al.; Li et al.) described the isolation of β-endorphin from human pituitary glands.

Today it is widely accepted that at least two, anatomically distinct opiate systems (neural and endocrine) exist within animals and man. Opiate peptides within the CNS appear to subserve a neural function since they have been localized within neurons and their distribution parallels that of opiate receptors. On the other hand, the pituitary gland contains the highest concentration of β-endorphin and is the major source for circulating β-endorphin suggesting that pituitary β-endorphin subserves

an endocrine function as a hormone.

Since the discovery of the enkephalins and β-endorphin, a fourth, extraordinarily potent opioid peptide called dynorphin has been isolated from porcine pituitaries (Goldstein et al., 1979) and subsequently found to be present within CNS neurons (Vincent et al., 1982). The discovery of this peptide generated considerable excitement since it contained within its sequence leu-enkephalin (Goldstein et al., 1979) and it was thought to be a precursor for this pentapeptide. Subsequent studies failed to prove this hypothesis and have shown instead that the enkephalins, β-endorphin and dynorphin are members of 3 separate opiate peptide groups each having different anatomical distributions within the CNS (Vincent et al., 1982; Watson et al., 1982) and different biosynthetic origins (see Rossier, 1982).

ANATOMICAL DISTRIBUTION OF β-ENDORPHIN AND RELATED PEPTIDES

After the discovery of β-endorphin's opiate actions, antisera became available for the study of its distribution in the brain and pituitary. Early on, the presence of the met-enkephalin sequence within β-LPH raised questions about the relationship between the enkephalins, β-endorphin and β-LPH. Were the enkephalins the 'real' opiate peptide or merely a breakdown product of β-endorphin? Immunohistochemical studies of these substances in brain showed that β-endorphin had a unique distribution, quite distinct from met-enkephalin and was therefore not just a precursor of enkephalin (Bloom et al., 1978; Watson et al., 1979). The anatomical studies of β-LPH and β-endorphin were in agreement in describing a system with a single major cell group in the periarcuate region of the hypothalamus with a

very widespread fiber system (see Fig. 1). These β-LPH/β-END fibers were visualized mainly in the medial hypothalamus, amygdala, basal septal nuclei, n. accumbens, periventricular thalamus, periaqueductal central gray area, and the locus coeruleus (Bloom et al., 1978; Bloch et al., 1978; Watson et al., 1977a). In comparison, met- and leu-enkephalin were found in 30-40 cell groups at every CNS level from the spinal cord to the limbic system nuclei (Watson et al., 1977b; Hokfelt et al., 1977; Simantov et al., 1977) and appeared to be contained within the terminals of short interneurons. Based on anatomical, biochemical, and lesion studies the enkephalins and β-LPH/β-END seem to be located in separate neuronal pathways. To date, there is no example of overlap in the cell bodies of the two systems although some overlap in their nerve terminal distribution exists.

Throughout the CNS the localization of opiate peptides is in parallel to the known distribution of opiate receptors. This finding suggests that CNS opiate peptides probably function as neurotransmitters or neuromodulators. In particular, the highest concentration of opiate receptors is in the periventricular-periaqueductal gray region, an area known to have a dense innervation from arcuate β-LPH/β-END nerve fibers and known to be important in stimulation-produced analgesia. Thus it appears that β-endorphin may be a neurotransmitter mediating the analgesia produced by electrical stimulation.

As the β-LPH/β-END and enkephalin pathways were being mapped in the CNS, pituitary studies demonstrated that β-LPH/β-END were located in corticotrophs of the anterior lobe and all cells of the intermediate lobe (Moon et al., 1973). Bloom et al. (1977) visualized β-LPH/β-END and adrenocorticotropin (ACTH) in the same two cell groups.

Figure 1. A schematic diagram illustrating the distribution of the

β-LPH/β-END/ACTH system in the rat central nervous system. (From

S.J. Watson and J.D. Barchas, 1979). β-LPH/β-END/ACTH peptides are

localized within a single major cell group in the periarcuate region

of the hypothalamus with axons projecting to areas in the medial hypo-

thalamus, the n. accumbens, the basal septal nuclei, the periventricular

thalamus, the periaqueductal central gray area and the locus coeruleus.

β-LPH/β-END/ACTH SYSTEM
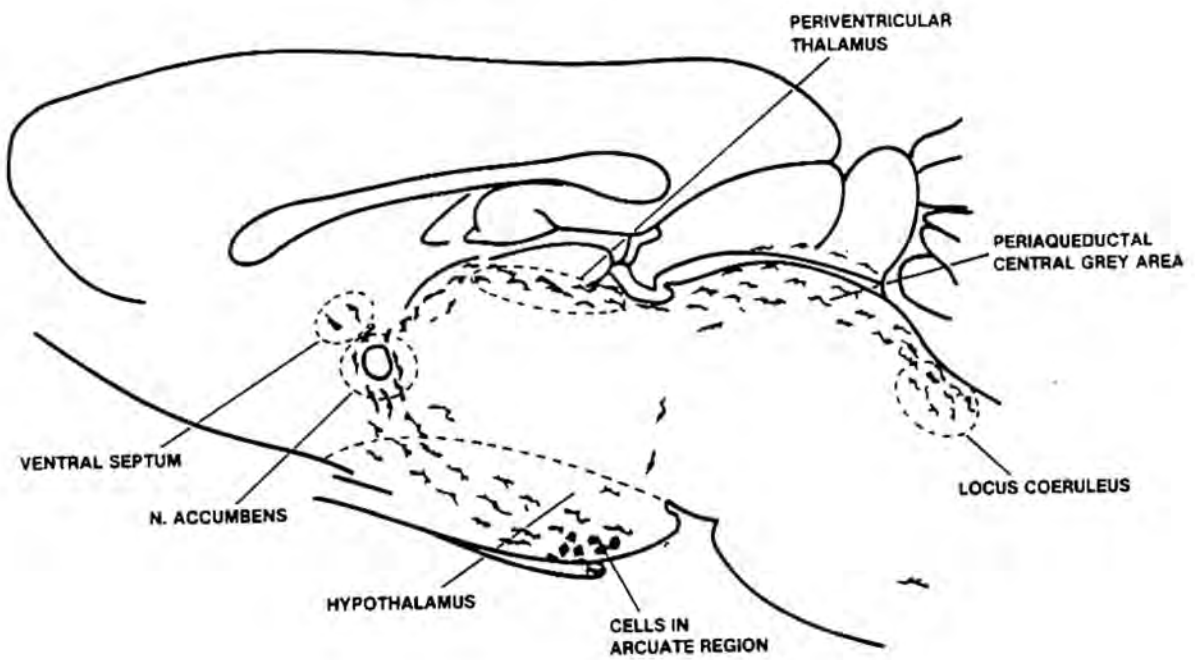
Figure 1

In electron microscopic studies of the pituitary, Pelletier et al. (1977) and Weber et al. (1978) were able to show that β-LPH and ACTH immunoreactivities were located within the same cells and secretory granules. This along with evidence suggesting parallel changes in pituitary β-LPH, melanotropin stimulating hormone (MSH) and ACTH led to the elegant studies of Mains et al. (1977) and Roberts and Herbert (1977) who demonstrated that pituitary ACTH, α-MSH, β-MSH and β-LPH (and therefore β-endorphin) share a common 31,000 dalton precursor. This 31K precursor was also called pro-opiocortin or pro-opiomelano-cortin (POMC).

The discovery of the 31K precursor in the pituitary rapidly lead to explorations of the brain for neuronally synthesized β-LPH/β-END, ACTH and MSH substances. At about the same time that β-endorphin and β-LPH were visualized in brain, Krieger and associates (1977) described the presence of ACTH immunoreactivity in the CNS (even after hypophys-ectomy). This finding led to a rather complex study by Watson et al. (1978) in which they found ACTH, β-endorphin and β-LPH staining located in precisely the same cells of the arcuate nucleus of the hypothalamus. Shortly thereafter, several laboratories reported finding α-MSH-like immunoreactivity in the brain (Jacobowitz et al., 1978; van Leewen et al., 1979) by light and electron microscopy. When parallel studies were carried out with β-endorphin, it was apparent that all the cells of the arcuate nucleus which contained β-endorphin also contained α-MSH along with all the other POMC peptides (Watson & Akil, 1980a,b). These studies stongly supported the hypothesis that ACTH, β-endorphin and β-LPH share a common cellular origin and biochemical precursor in pituitary and brain.

β-Endorphin and the enkephalins have also been found outside the pituitary gland and CNS. In the periphery, the distribution of both opiate peptides has been found to be in close proximity to opiate receptors. Opiate compounds have long been recognized as having potent effects on gastrointestinal function, and the guinea pig ileum longitudinal smooth muscle preparation has been used as a bioassay for opiate activity. Therefore, it is not surprising to find a rich enkephalinergic innervation of the gastrointestinal tract (Hughes et al., 1977) as well as high concentrations of β-endorphin-like immunoreactivity within these tissues (Orwoll & Kendall, 1980). Also, β-endorphin-like immunoreactivity has been extracted from pineal gland, pancreas and adrenal gland (Vuolteenaho et al., 1980). In addition, immunoreactive β-endorphin has been found in CSF and plasma (Nakae et al., 1980). Extracts of human placental tissue also contain significant amounts of β-endorphin-like peptides (Houck et al., 1980) as also does amniotic fluid (Gautray et al., 1977), suggesting that these peptides may function as a natural antidote for the pain and stress of parturition.

BIOSYNTHESIS OF β-ENDORPHIN AND RELATED PEPTIDES

While several lines of evidence had suggested that ACTH, β-LPH and β-endorphin came from a common precursor (Lowry et al., 1976), the studies of Mains et al. (1977) and Roberts and Herbert (1977) were the first to clearly demonstrate that relationship. Mains, Eipper and co-workers (1975) used the pulse-chase method with a mouse pituitary tumor line and immunoprecipitation by antisera against ACTH and β-endorphin. In this series of studies they demonstrated that ACTH, β-LPH and β-endorphin all came from a common precursor with an apparent

molecular weight of 31,000 daltons. Roberts and Herbert (1977), using

the same tissue source, extracted the messenger RNA from cells rich in

ACTH and β-endorphin and identified the precursor protein among the

translation products. They also suggested the possible arrangement

of the peptides within the precursor. Starting from the amino terminal

region and moving toward the carboxy terminus, the first fragment had

a molecular weight of 16,000 daltons, the next structure was $ACTH_{1-39}$,

followed by the 91 residues of β-LPH of which the last 31 residues

constituted $\beta-endorphin_{1-31}$.

More recently, Nakanishi et al. (1979) confirmed the findings of

Mains et al. (1977) and Roberts and Herbert (1977) using DNA cloning

and nucleotide sequence analysis. Using these techniques, Nakanishi

et al. (1979) defined the precise locations of ACTH and β-LPH in the

precursor protein and predicted the amino acid sequence of its

remaining portion. The protein-structural studies on the ACTH/β-END

precursor also revealed the presence of a signal sequence preceeding the

amino terminal region suggesting that it follows the traditional

structure of a prohormone. In addition, it was shown that each of

the known peptides were separated from each other by dibasic amino

acids suggesting post-translational peptide cleavage at these sites.

Thus it appears that the POMC precursor is synthesized as a prohormone

following the classical protein biosynthetic pathways and then is

cleaved into its smaller peptides.

In addition, it is known that POMC can undergo further post-

translational processing involving glycosylation and amidation at

several sites along its structure and further modification of its

cleavage products by way of acetylation. These post-translational

events appear to occur to different extents in various tissues and appear to represent important mechanisms for regulating end-product bioactivity.

Several laboratories have sought to determine the precise sequence of events involved in the post-translational processing of the precursor to its many known peptide products. Using the pulse-chase technique in which a labeled amino acid is incorporated into the precursor (pulse) and then followed through its conversion to product with incubations of unlabeled amino acids (chase) over time, Eipper and Mains (1978) and Herbert et al. (1980) have shown that β-LPH is the first to be cleaved away from the intact precursor. This leaves the amino terminal 16K fragment and $ACTH_{1-39}$ which are subsequently cleaved. Further processing converts β-LPH to β-endorphin$_{1-31}$ (Liotta et al., 1978). In the anterior lobe of the pituitary, $ACTH_{1-39}$ is found along with β-LPH and relatively little β-endorphin$_{1-31}$ (Eipper and mains, 1980). The pituitary intermediate lobe, on the other hand, processes the precursor further to yield α-MSH (N-acetyl $ACTH_{1-13}$) (O'Donohue et al., 1979; Crine et al., 1979), corticotropin-like intermediate lobe peptide (CLIP; $ACTH_{18-39}$) and modified forms of β-endorphin with little or no ACTH or β-LPH detectable (Mains & Eipper, 1979, 1981, Eipper & Mains, 1981, Liotta et al., 1981; Akil et al., 1981a) (see Fig. 2).

Smyth et al. (1978, 1979) were the first to isolate modified forms of β-endorphin from pituitary extracts which they identified as β-endorphin$_{1-31}$, β-endorphin$_{1-27}$ and their α-N-acetyl derivatives on the basis of chromatographic behavior and amino acid analysis. Liotta et al. (1981) further substantiated these results by demonstrating that cultured dispersed rat intermediate lobe cells

Figure 2. A schematic diagram illustrating the proteolytic

processing of the 3IK precursor, pro-opiomelanocortin (POMC),

in different pituitary tissues. (From B.A. Eipper and R.E.

Mains, 1980).

31 K precursor

oligosaccharide

Biosynthetic intermediate + β LPH

16 K fragment + ACTH   γ LPH + β-endorphin

smaller peptides   αMSH   CLIP   smaller peptides   met-enkephalin α-endorphin

Intermediate Pituitary, but not Anterior Pituitary or Tumor Cells
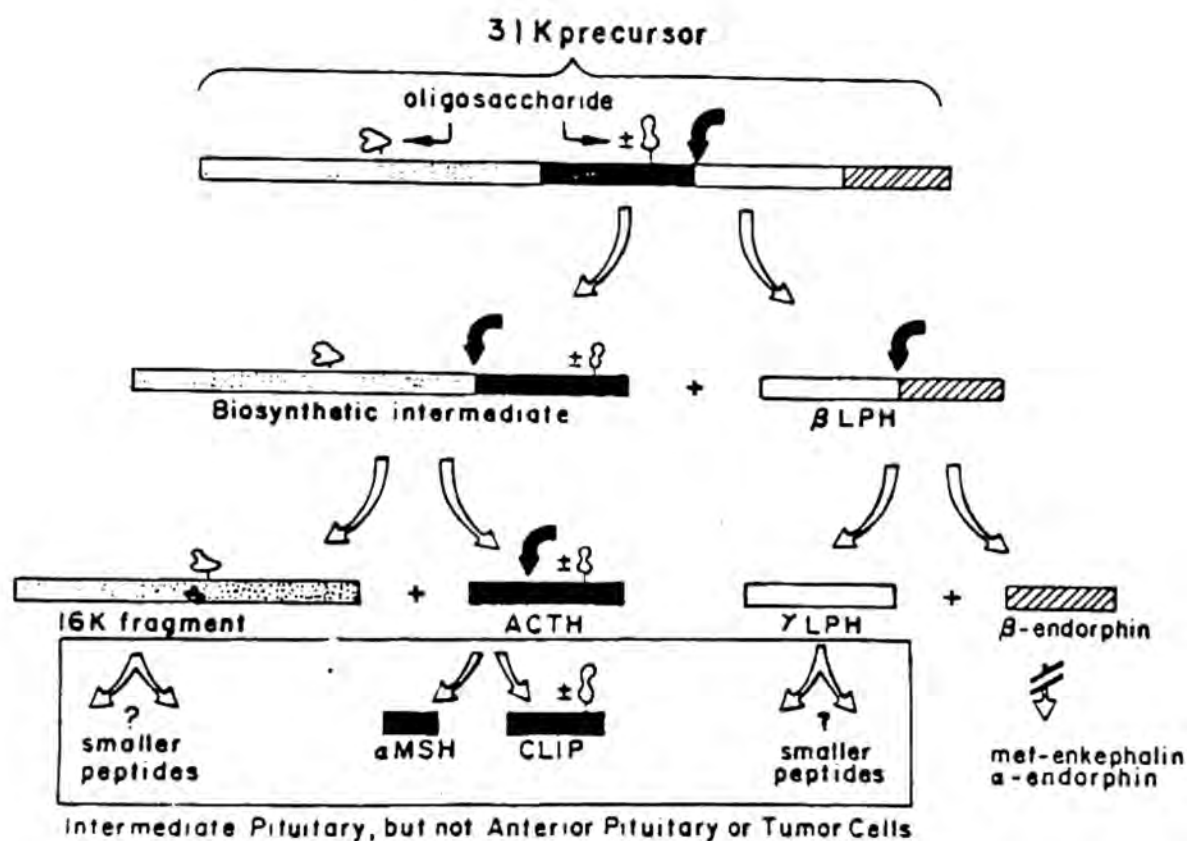
Figure 2

synthesize and release the four modified forms of β-endorphin under basal and stimulatory conditions. In contrast, virtually all of immunoreactive β-endorphin extracted from and secreted by anterior lobe cells was β-endorphin$_{1-31}$.

The observation that β-endorphin$_{1-31}$ can be further cleaved and modified by acetylation raises important questions about the physiologic roles of these peptides. β-endorphin$_{1-31}$ has been shown to be 20-50 times more potent than morphine in opiate bioassay systems (Li et al., 1976b) and in producing analgesia (Loh et al., 1976). Deakin et al. (1980) and Akil et al. (1981b) have shown that N-terminal acetylation and/or carboxy-terminal proteolysis of four amino acids of β-endorphin$_{1-31}$ leads to almost a complete loss of analgesic potency and a diminished ability to bind to opiate receptors. It becomes critical therefore to determine whether the β-endorphin-like immunoreactivity being measured is β-endorphin$_{1-31}$, the potent opiate, or its shorter, modified forms which exhibit little or no known biological activity (antibodies developed against β-endorphin$_{1-31}$ will recognize to some extent its inactive forms). Fortunately, biochemical tools are available for the separation of β-endorphin from its parent molecule (gel filtration chromatography), as well as the resolution and identification of β-endorphin$_{1-31}$ from its smaller and modified forms (HPLC and ion exchange chromatography).

Most of the POMC biosynthetic studies have been carried out on pituitary POMC since the brain contains small amounts of this peptide. However, work by Liotta et al. (1980) have demonstrated the biosynthesis of POMC in cultured rat hypothalamic cells with the resultant production of ACTH, α-MSH, β-LPH and β-endorphin-like peptides.

Interestingly, there is evidence to suggest that brain POMC processing resembles pituitary intermediate lobe POMC processing in that it has a preponderance of smaller over larger peptide products, e.g., mostly β-endorphin-sized immunoreactivity with little β-LPH or larger POMC-sized immunoreactivity (Fratta et al., 1980). In addition, it synthesizes α-MSH as opposed to ACTH (Jacobowitz & O'Donohue, 1978; Watson & Akil, 1980a). Based on such evidence, one would expect to find the smaller and acetylated forms of β-endorphin$_{1-31}$ to be present in brain as well. Preliminary evidence (Zacharian & Smyth, 1979) suggests that there is a gradient in brain with the cell bodies in the arcuate nucleus containing mostly the opiate-active β-endorphin$_{1-31}$, while the terminal regions appear to store the N-acetylated forms. This would suggest that the peptide is processed and further modified either during transport or storage.

The biological significance of the smaller molecular forms of β-endorphin and the acetylated peptides are presently unknown. It has been suggested (Zakarian & Smyth, 1979) that since the acetylated forms of β-endorphin are formed concomitantly with α-MSH, then the acetylation reaction which imparts biological activity to α-MSH and eliminates opiate activty of β-endorphin may provide a means for selective activation of different regions of the endorphin prohormone. In general it is believed that the smaller, acetylated and non-acetylated β-endorphin fragments represent inactivated forms of β-endorphin$_{1-31}$ that are stored intracellularly. Furthermore, in vivo this selective processing and inactivation may represent mechanisms by which the normal levels of opiate activity are regulated within the pituitary gland and CNS.

OPIATE RECEPTORS

Interestingly, demonstration of the existence of opiate recep-
tors preceeded the discovery of the endogenous opiate peptides. In
fact, it was the existence of opiate receptors that spurred efforts to
isolate the endogenous ligands for the morphine binding sites. Since
the discovery of opiate receptors in 1971 (Goldstein et al.), there
has been widespread interest in determining their exact localization,
number and physiochemical characteristics, as well as their physio-
logic and pharmacologic functions. As a result of these various stud-
ies, evidence has accumulated to suggest that multiple receptor types
exist for the different opiate peptides and that their distribution
parallels that of β-LPH/β-END and enkephalin within the brain and in
the periphery.

Within the CNS, opiate receptor distributions correspond to
a great extent to certain pathways in the brain that are known to
mediate pain suggesting that these receptors and opiate-like subs-
tances (whether exogenous or endogenous) may be related to pain and
its transmission in the nervous system. Areas known to have a large
number of opiate receptors both in primates and rats are the hypothala-
mus, thalamus, the periventricular-periaqueductal gray, parts of the
lateral midbrain including the lateral reticular nucleus, substantia
nigra and the red nucleus (Kuhar et al., 1973; Snyder, 1975). Recep-
tors are also found in the spinal cord, especially in the dorsal horn
(Fields et al., 1980) and in the sensory (olfactory, visual, auditory
and somatic) and limbic systems (Herkenham and Pert, 1980) suggesting

that these receptors might play a role in the control of sensory and

limbic information processing. Few receptors are found in the pons-

medulla and the posterior cortex or cerebellum, with the lowest numbers

found in the hippocampus (Messing et al., 1980).

There are areas outside the CNS which have been shown to con-

tain opiate receptors. These include the guinea pig ileum (Pert &

Snyder, 1973), mouse vas deferens (Henderson et al., 1972), pancreas,

gastrointestinal tract, and adrenal medulla. Recently, opiate receptor

binding activity has been located on lymphocytes (Hazum et al., 1979a)

suggesting that β-endorphin may serve as a link between neuroendocrine

and immune systems.

The generally accepted hypothesis of opiate receptor heterogen-

eity is largely based on evidence from biochemical studies showing a

differential affinity of enkephalins and morphine to receptor sites

in brain membranes. More recently evidence from both in vitro (Lord et

al., 1976; Chang et al., 1979) and in vivo (Martin et al., 1976) studies

has suggested the existence of more than one opiate receptor type.

There are opiate receptors which appear to prefer enkephalins (so-called

delta sites; δ receptor), receptors which prefer morphine (so-called

mu sites; μ receptor) and receptors which prefer a class of unusual

synthetic opiates such as ethylketocyclazocine (called kappa receptors)

and SKF-10047 (called sigma receptors). Although studies using smooth

muscle preparations have differentiated the kappa and sigma receptors,

binding studies in brain membranes have been less successful in differ-

entiating these receptor types. So far only the existence of mu and

delta receptors has been well accepted and considerable controversy

still exists over whether the other proposed opiate receptors represent

physiologically distinct receptors in vivo.

A striking observation is that β-endorhpin is active to varying degrees at all these proposed receptor sites. In particular, β-endorphin has high and equal affinity for both the mu and delta receptor (Lord et al., 1976;1977; Hazum et al., 1979b). In general, no receptor is specific for just one ligand and considerable overlap between opiate ligand binding occurs. These observations suggest that the different classes of opiate receptors may actually respresent interconverting conformational states of the same macromolecule rather than separate receptor types. Although this hypothesis accounts for a great deal of well-documented data not easily explained by the existence of distinct mu and delta sites, this hypothesis continues to be challanged by the overwhelming evidence supporting the existence of multiple opiate receptors.

Despite the increasing number of opiate receptors which have been differentiated by in vitro binding studies and anatomic distributions, possible physiologic functions have been elucidated primarily for the mu and delta receptors. Evidence from structure-activity studies has suggested that analgesia is mediated by the mu receptor, whereas emotional behavior (behavioral epileptic seizures) and respiratory depression are primarily mediated by the delta receptor (Waterfield et al. 1979; Kosterlitz et al., 1980; Urca et al., 1978; Snyder, 1980; Pasternak and Hahn, 1980). In support of these findings, anatomically the mu receptors (but not the delta receptors) are localized in CNS areas known to be involved in the regulation of sensory input such as pain perception (Herkenham and Pert, 1980; Goodman et al., 1980). On the other hand, delta receptors are most concentrated in the frontal cere-

bral cortex and areas involved in the regulation of cardiorespiratory function (Goodman et al., 1980).

In summary, the parallel distribution of opiate receptors and endogenous opiates within the CNS and periphery suggests that endogenous opiates function as neurotransmitters or neuromodulators. Moreover, highest concentrations of opiate receptors and their endogenous ligands are found in areas of the brain known to be involved in analgesia. Thus it appears that opiate receptors and opiate peptides are related to pain and its transmission in the CNS. It is important to note that pituitary β-endorphin can serve as an endogenous ligand for peripheral and CNS opiate receptors. Thus, the location and action of opiates on opiate receptors within the CNS or in the periphery provides insight into the possible physiological functions for pituitary and blood-borne β-endorphin.

PHARMACOLOGICAL, PHYSIOLOGICAL AND BEHAVIORAL STUDIES WITH β-ENDORPHIN

Following the isolation and identification of opioid peptides in brain and pituitary, studies were undertaken to determine the physiological functions of these novel peptides. The anatomical localization of the endogenous opiates and opiate receptor in areas known to be involved in pain transmission and analgesia suggested that these peptides play an important role in pain adaptation. Earliest evidence for the existence of an endogenous analgesia system was the observation of Reynolds (1969) that electrical stimulation of certain discrete brainstem sites in rats produces profound analgesia without a general behavioral depression - a phenomenon now commonly referred to as stimulation - produced analgesia (SPA). Since the initial report by Reynolds,

several workers have demonstrated the potent analgesic effects produced by electrical stimulation of discrete areas of the medial diencephalon and brainstem in the cat (Liebeskind et al., 1973), monkey (Goodman and Holcombe, 1975) and rat (Mayer et al., 1971). Furthermore, intractable clincal pain states in humans, in addition to normal pain perception, can be block by electrical stimulation of the periaqueductal and periventricular gray matter (Hosobuchi et al., 1977; Richardson and Akil, 1977). In all these studies, SPA has been shown to be most effective at sites known to have a high concentration of endogenous opiates and opiate receptors suggesting that electrical stimulation of endogenous opiate peptide release and subsequent interaction with opiate receptors leads to the production of behavioral analgesia. The involvement of endogenous opiates in SPA is further supported by the finding that naloxone, an opiate antagonist, blocks SPA in animals (Mayer et al., 1977; Akil et al., 1976) and man (Hosobuchi et al., 1977) and CSF levels of β-endorphin-like immunoreactivity increase during pain relief following stimulation of the periaqueductal gray in humans (Hosobuchi et al., 1979).

Pharmacologic studies with β-endorphin have shown that this peptide, like morphine, is a very potent analgesic after central administration into laboratory animals (Feldberg and Smyth, 1976; Loh et al., 1976; Nemeroff et al., 1979) and man (Oyama et al., 1980) but β-endorphin is at least 20 times more potent than morphine on a molar basis. Peripheral administration of β-endorphin has been reported to require higher doses (in mice) to produce analgesia (Tseng et al., 1976). In all cases, however, the analgesia produced by β-endorphin, like morphine, was reduced by naloxone suggesting that the event in-

volves opiate receptors. The observation that intravenous administra-
tion of β-endorphin can produce analgesia strongly suggests that
pituitary β-endorphin, which is the major source for circulating β-end-
orphin, can produce analgesia possibly by binding to CNS opiate recep-
tors.

Subsequent research into the anatomy, physiology and pharmacology
of opiate analgesia (OA) and SPA revealed many common features suggesting
that both responses are mediated via common mechanisms. For example,
the most effective sites for SPA and OA by intracerebral injection
overlap considerably (Lewis and Gebhart, 1977), and there is cross-
tolerance between OA and SPA (Mayer and Hayes, 1975). Most importantly,
however, are the findings that naloxone reverses both OA and SPA.
Therefore, if OA and SPA act through the same intrinsic mechanism, it
is likely that endogenous opiates and opiate receptors are involved
in the pain suppression system.

Present evidence indicates that OA and SPA activate an efferent
brainstem system that suppresses pain transmission at spinal cord
levels. Based on a review of anatomical, physiological and pharmaco-
logical evidence, Basbaum and Fields (1978) have proposed a model for
endogenous pain control involving opiates and biogenic amines. Basbaum
and Fields have proposed that this pain suppression system is organized
at three levels of the neuraxis: midbrain, medulla and spinal cord.
Activation of neurons in the midbrain periaqueductal gray matter (PAG)
(by electrical stimulation, opiates and possibly psychological factors)
excites neurons of the rostral medulla, some of which contain serotonin.
The medullary neurons, in turn, project to and specifically inhibit
the firing of trigeminal and spinal substance P containing neurons

which carry pain information from receptors in the periphery. The pain-transmitting neurons which are activated by the substance P primary afferent neuron project to supraspinal sites and indirectly contact the cells of the descending analgesia system in the PAG and medulla, thus establishing a negative-feedback loop.

At the spinal cord level, it appears that descending brainstem (serotonergic) inhibition of pain transmission is mediated through an enkephalinergic interneuron located in the dorsal horn. This is based on the extensive anatomical overlap between serotonergic nerve terminals, opiate receptors and enkephalins in the dorsal horn of the spinal cord, and the observation that local application of opiates inhibits nociceptive responses of various types of dorsal horn neurons (Kitahata et al., 1974) and naloxone completely reverses serotonin-mediated SPA (Oliveras et al., 1977).

In addition to a direct effect on pain transmission at the spinal cord level, endogenous opiates, particularly β-endorphin, may modify pain perception at the level of the limbic lobe. Arcuate β-endorphin neurons project to limbic structures known to be involved in the affective component of pain, and the regional distribution of opiate receptors suggests some role related to limbic system function. Thus, it is probably misleading to assume that opiate receptors exclusively mediate analgesia.

Other possible physiologic functions for central β-endorphin include a role in cardiovascular function (see Holaday, 1983). Indeed, opiate receptors are densely distributed in the brainstem and hypothalamus in close proximity to cardiovascular centers. Generally speaking, morphine has been shown to produce effects upon brainstem cardiovascular

centers which include increased parasympathetic and decreased sympathe-
tic tone resulting in a depression of both heart rate and blood pres-
sure. The central or peripheral administration of opioid peptides has
been shown to produce potent and variable cardiovascular responses
depending on the dose, injection site, species, presence and type of
anesthetic used and particular opioid used. The fact that different
opioid ligands produce qualitatively different cardiovascular effects
indicates that more than one opiate receptor subtype may be involved,
possibly at different anatomical sites.

Compelling evidence that endogenous opiate systems help regulate
cardiovascular function derives from the results of opiate antagonist
injections in various physiologic and pathophysiologic states. The
finding that naloxone and other opiate antagonists reverse the hemo-
dynamic effects of circulatory shock (both hemorrhagic and endotoxic)
suggests that endorphins are involved in this phenomenon. Similar
evidence has suggested a role for endorphin in the pathophysiology of
spinal cord injury, baroreceptor reflex responses, orthostatic hypoten-
sion, anesthetic hypotension and possibly in essential hypertension,
as well as cerebral vascular disorders. These findings do not pre-
clude the possible involvement of peripheral β-endorphin of pituitary
origin in these pathophysiologic states, and, in fact, blood levels
of β-endorphin have been reported to be elevated under most of these
circumstances.

The presence of high concentrations of opiate receptors and
endorphin in the solitary nuclei and area postrema of the medulla, areas
known to be involved in ventilatory control, suggest a role for endo-
genous opiates in respiratory function. It is well documented that

morphine and other opiates cause respiratory depression. Similarly, opioid peptides have been shown to depress respiration after central (Moss and Friedman, 1978; Florez et al., 1980) but not peripheral administration (Sitsen et al., 1982). A role for endogenous opiates in human respiratory function is difficult to elucidate since the placebo effect on ventilatory control is pronounced. However, endogenous opiates have been implicated in such disorders of ventilatory control as sudden-infant-death syndrome, apnea, and chronic obstructive pulmonary disease based on studies with naloxone (see Chernick, 1981). Clearly, more direct evidence involving actual administration of β-endorphin and/or measurment of plasma β-endorphin levels in humans is necessary to better assess a role for central and/or peripheral (pituitary) β-endorphin in the pathophysiology of respiratory disorders.

Other central effects of β-endorphin including feeding, grooming, sexual behavior, and temperature regulation suggest that these effects are mediated at the hypothalamic level. In rats, central administration of β-endorphin has been shown to elicit abnormal male sexual behavior (Meyerson and Terenius, 1977), excessive grooming (Gispen et al., 1976), and stimulation of food intake (Grandison & Guidotti, 1977). Relatively low doses of opiates which result in only mild analgesia produce elevations in body temperature, whereas higher doses progessively lead to a lowering of body temperature (Holaday, et al., 1978). All of the above effects of β-endorphin are presumably central, with the exception of temperature and food intake regulation, which may involve pituitary β-endorphin as well. In addition, the above effects of β-endorphin are presumably mediated by opiate receptors since they can be reversed by naloxone. It is important to note

that, in view of the potent effects of the opioid peptides on pituitary
hormone release, it may be premature to conclude that all of these
behavioral manifestations are direct effects.

PITUITARY β-ENDORPHIN AND ITS POSSIBLE FUNCTIONS

The greatest store of β-endorphin in the mammalian body is in
the pituitary gland (Fratta et al., 1979), which is also the major source
for circulating β-endorphin-like immunoreactivity (Guillemin et al.,
1977; Mueller, 1980). Current evidence suggests that pituitary and CNS
β-endorphin represent two distinct opiate systems (endocrine versus
neural, respectively) which function together in the production of
analgesia and other behavioral adaptations to pain and stress.

Pituitary β-endorphin most likely functions as a hormone since
it is released into blood in response to physiologic and pharmacologic
stimuli, and its release appears to be regulated by physiological
mechanisms. In addition, β-endorphin has been shown to be comparatively
stable in blood (Houghten et al., 1980) and to be active as an analgesic
when injected intravenously (Tseng et al., 1976). However, target
site(s) and thus the physiological functions for blood-borne β-endorphin
remain to be clearly defined. It is likely that target site(s) for
pituitary β-endorphin exist both in the periphery and CNS since
intravenously administered β-endorphin has been shown to penetrate
the blood-brain barrier at physiological concentrations (Rapoport et
al., 1980). It is also possible for pituitary β-endorphin to gain
access into the CNS by either direct retrograde transport from the
pituitary to the hypothalamus via the portal vessels (Bergland and
Page, 1978; Mezey and Palkovitz, 1982), or from the blood through more

permeable regions of the blood-brain barrier (Houghten et al., 1980; see Meisenberg and Simmons, 1983).

A large body of literature supports a role for pituitary β-endorphin in the physiologic response to pain and stress. In humans, two events associated with pain and stress, surgery and childbirth, have been shown to elevate plasma levels of β-endorphin-like immunoreactivity (Dubois et al., 1981; Csontos et al., 1979). Physical stress and pain have been shown to be the most potent stimuli for pituitary β-endorphin release in animals (Guillemin et al., 1977; Mueller, 1981). Other physical stressors which appear less noxious to the animal (footshook, ether stress, exposure to warm and cold ambient temperature) produce comparably lower increases in plasma levels of β-endorphin-like immunoreactivity (Mueller, 1981) suggesting that the magnitude of the plasma β-endorphin response is directly related to the degree of stress or trauma experienced. Under most conditions of pain and stress, a behavioral analgesia is observed in laboratory animals which also appears to be at least parially mediated by pituitary β-endorphin. Hypophysectomy (Bodnar et al., 1979; Lewis et al., 1981) which removes the source of circulating endorphins, and glucocorticoids (Guillemin et al., 1977; Lewis et al., 1980) which inhibit pituitary β-endorphin release, both attenuate stress-induced analgesia in a manner similar to naloxone (Amir and Amit, 1978). Although the plasma levels of β-endorphin required to produce analgesia in rodents are higher than that usually observed in stressed animals (Tseng et al., 1976), it is possible that the various experimental treatments which release pituitary β-endorphin into general circulation also increase the delivery of pituitary β-endorphin, in relatively high concentration,

directly into the brain via the hypophysial portal blood system and thus affect nociception at the CNS level. Evidence to date suggests that pituitary β-endorphin's role in endogenous pain control is most likely mediated through its direct actions on pain regulatory pathways of the brain and spinal cord, as well as, its ability to desensitize peripheral pain receptors (Ferreira, 1981; Hughes, 1981).

In addition to being involved in the analgesia associated with stress, pituitary and CNS β-endorphin may serve together to regulate neural and endocrine functions that play a role in the elaboration of adaptive behavior to stress (see Amir et al., 1979). β-Endorphin has been shown to exert a trophic influence on the adrenal medulla and cortex as well as the pancreas, suggesting that circulating β-endorphin may be part of a physiologic mechanism that mediates adrenaline, corticosterone and glucagon release in response to stress. Other evidence suggests that endogenous opiates may also be involved in the elaboration and expression of the emotional responses to stress.

The high concentration of β-endorphin peptides in both the medial basal hypothalamus and pituitary suggested that these peptides may play a role in neuroendocrine function. Subsequent studies have established that endogenous opiates, like morphine, can alter pituitary secretions and that they are involved in endocrine function (see Meites et al., 1979; Holaday and Loh, 1979). In general, the central admini-stration of β-endorphin has been shown to produce a rapid increase in the release of prolactin, growth hormone, ACTH, MSH and antidiuretic hormone and a decrease or no change in the gonadotropins and thyrotro-pin. Little is yet known about the mechanisms by which the endogenous opiates influence endocrine function, but most of the work reported

thus far indicates that their endocrine effects are exerted via the CNS. These findings further support the conclusion that pituitary, and thus circulating β-endorphin, can indeed influence CNS function.

Other possible functions for pituitary and blood-borne β-endorphin include a role in the immune response and this too may be related to stress adaptation. β-Endorphin has been shown to enhance lymphocyte proliferation in response to T cell mitogens (Gilman et al., 1982). This effect, however, does not appear to be mediated through an opiate receptor. It has also been shown that human leukocyte interferon binds to opiate receptors in vitro and produces potent β-endorphin-like opioid effects including analgesia in vivo (Blalock and Smith, 1981). These new and interesting studies suggest a functional link between the endocrine and immune systems involving the endogenous opiates.

Finally, pituitary β-endorphin has also been implicated in food/water intake regulation (see Morley, 1980), heat adaptation and thermoregulation (Holaday et al., 1978a, b). In addition, circulating β-endorphin appears to be involved to some extent in peripheral cardiovascular function especially in the development of hypotension associated with circulatory shock due to hemorrhage or endotoxemia (see Holaday 1983). Endotoxemia and hemorrhage have been shown to elevate plasma levels of β-endorphin (Carr et al., 1982; Lang et al., 1982), and elevated plasma levels of β-endorphin have been shown to produce a transitory bradycardia and hypotension in conscious rats and monkeys (Holaday et al., 1982, Cuthbert et al., 1983). Thus, it is likely that circulating β-endorphin may play a pathophysiological role in the development of certain forms of circulatory shock. Although evidence also suggests an important role for CNS β-endorphin in the regu-

lation of CNS cardiovascular function, it is possible that circulating β-endorphin released from the pituitary may gain access to CNS cardiovascular centers to modulate cardiorespiratory function. In addition, although little evidence is available, direct peripheral opiate effects upon the heart or vascular beds may also be a possible site of action for blood-borne β-endorphin. Unfortunately, none of the demonstrated effects for circulating β-endorphin have proven physiologic importance. In summary, of all the functions for pituitary β-endorphin which have been proposed, the evidence suggesting pituitary β-endorphin involvement in endogenous mechanisms of analgesia is the most compelling and has been the most widely studied.

NEUROENDOCRINE REGULATION OF PITUITARY β-ENDORPHIN PEPTIDES

An understanding of the neuroendocrine regulation of pituitary β-endorphin secretion is based on a knowledge of the anatomy of the gland and its relationship to the hypothalamus. Anatomically, the pituitary is divisible into two distinct portions: the anterior pituitary (adenohypophysis) and the posterior pituitary (neurohypophysis). Between these lobes exists a small, relatively avascular zone called the pars intermedia. In lower animals, the pars intermedia is highly developed and functions to secrete MSH in response to the amount of light or other environmental factors to which the animal is exposed to. In humans, the pars intermedia is almost absent except during pregnancy and fetal life. Since the pituitary gland is situated at the base of the brain and has some anatomical connection with the hypothalamus, it is not surprising that almost all secretion by the pituitary gland is controlled by either hormonal or neural signals

from the hypothalamus.

The adenohypophysis (pars intermedia and pars distalis) is composed of several distinct cell types which relate to the secretion of specific hormones. It is now known that β-endorphin is synthesized and secreted together with ACTH from pars distalis corticotrophs and with α-MSH from pars intermedia melanotrophs. Accordingly, circulating levels of β-endorphin-like immunoreactivity parallel the release of ACTH and α-MSH from these two lobes. A variety of stresses such as limb fracture, foot shock and immobilization have been shown to elevate circulating levels of both β-endorphin and ACTH (Guillemin et al., 1977). In addition, physiologic and phamacologic manipulations known to affect pituitary ACTH secretion (e.g. adrenalectomy, metyrapone and glucocorticoids) similarly influence β-endorphin secretion (Guillemin et al., 1977; Hollt et al., 1978). Parallel changes in blood-borne β-endorphin and α-MSH are more difficult to demonstrate since few stimuli are known to selectively activate pars intermedia secretion. Since pars distalis β-endorphin and ACTH and pars intermedia β-endorphin and α-MSH appear to share similar secretory patterns, it can be inferred that these peptides, likewise, share common regulatory mechanisms. Evidence to date indicates that these peptides do share common regulatory mechanisms and that the regulation of pars distalis secretion is different and separate from that of the pars intermedia.

Pars distalis β-endorphin secretion, like ACTH secretion, is most likely regulated by humoral factors secreted by hypothalamic neurosecretory cells (hypothalamic hormones) or released from its possible target organs. These hormones reach pars distalis cortico-trophs by way of the hypothalamic-hypophysial (pituitary) portal blood

system. The synthesis and/or release of hypothalamic hormones into the portal blood vessels is regulated, in turn, by neuronal pathways mostly originating in the mesencephalon and lower brain stem. In contrast, pars intermedia β-endorphin, like α-MSH, is most likely regulated by the neurons which directly innervate this lobe since the pars intermedia has little apparent connection with the portal blood system and is relatively avascular.

Anterior lobe ACTH secretion is balanced between a stimulatory signal from the hypothalamus via the hypothalamic hormone corticotropin-releasing factor (CRF) and an inhibitory feedback signal from adrenal cortical glucocorticoids. CRF has been shown also to be a potent stimulator of β-endorphin-like immunoreactivity from anterior lobe cells in vitro (Vale et al., 1979, 1983) and in vivo (Rivier et al., 1982). Furthermore, glucocorticords have been shown to selectively inhibit the release of β-endorphin-like immunoreactivity from the anterior lobe both in vitro (Vale et al, 1979; 1983) and in vivo (Mueller et al., 1981). Other weaker secretagogues that exclusively stimulate the release of β-endorphin-like immunoreactivity from cultured anterior lobe cells are lysine vasopressin, oxytocin, norepinephrine and angiotension II (Vale et al., 1983).

In contrast to the anterior lobe, intermediate lobe (pars intermedia) β-endorphin secretion appears to be regulated by direct neural contacts rather than by a humoral mechanism. Consequently CRF, except at high concentrations, and other circulating hormones (Vale et al., 1979; 1983) appear to have little influence on the physiologic regulation of intermediate lobe β-endorphin secretion. Since dopaminergic and noradrenergic neurons innervate the pars intermedia, it

is not surprising that dopamine and β-adrenergic agents have been shown to have a direct effect on intermediate lobe β-endorphin secretion. Dopamine has been shown to inhibit (Vale et al., 1979; Vermes et al., 1980), whereas, β-adrenergic receptor agonists have been shown to stimulate (Pettibone and Mueller, 1982) the secretion of β-endorphin-like immunoreactivity from the pars intermedia in vivo. In vivo dopamine neurons have been shown to inhibit pituitary MSH (Tilders and Smelik, 1977) and immunoreactive β-endorphin secretion (Giraud et al., 1980; Farah et al., 1982).

Evidence to date suggests that the neurotransmitter systems that regulate pituitary ACTH secretion, likewise, influence pars distalis β-endorphin secretion. Although catecholaminergic and serotonergic neurons have been reported to inhibit or have no effect on ACTH release (Ganong et al., 1976; Tilgedy and Vermes, 1973), the majority of evidence supports a stimulatory role for these biogenic amines in the release of CRF-mediated ACTH and β-endorphin (Vale et al., 1978; Fuller, 1981, Pettibone and Mueller, 1982; Bruni et al., 1982). Since catecholaminergic and serotonergic neurons are not known to innervate the pars distalis, their control over corticotrophic β-endorphin secretion is most likely mediated through CRF or some other hypothalamic β-endorphin releasing factor(s).

Thus, in summary, the pars distalis and pars intermedia represent two separate sources of pituitary β-endorphin. Furthermore, the regulation of pars distalis and pars intermedia β-endorphin secretion, like the biosynthetic processing of POMC, is different for these two lobes of the adenohypophysis. Whereas pars distalis β-endorphin secretion appears to be regulated by a humoral mechanism, pars inter-

media β-endorphin is primarily regulated by the neurons which innervate this lobe. Ultimately, CNS neurotransmitter pathways carrying inputs from various portions of the brain govern the release of hypothalamic hormones and possibly control the neurons which directly influence pituitary secretion.

SEROTONIN NEURONS AND THE REGULATION OF PITUITARY β-ENDORPHIN

Serotonin Neurons and Analgesia:

A growing body of literature has accumulated to support an important role for serotonin neurons in endogenous pain control. Since endogenous opiates are known to be involved in mechanisms of analgesia, then the possibility of serotonergic regulation of endogenous opiates is likely and of particular interest to study.

The first evidence that serotonin neurons play a role in the behavioral response to noxious stimuli was made by Tenen in 1967. He observed that following the administration of para-chlorophenylalanine (PCPA), a drug which reduces serotonin biosynthesis, rats had a shorter latency response to a painful stimulus. Moreover, this hyperalgesia could be reversed by treating the animals with doses of serotonin precursors which restored serotonin content and presumably serotonergic function. These basic findings have been repeated in subsequent experiments carried out in other laboratories (see Messing and Lytle 1977). In addition, similar alterations in nociception have been reported in humans treated with PCPA (Sicuteri et al., 1973). The majority of the evidence indicates that pharmacological reductions in brain serotonin concentrations are associated with a behavioral hyperalgesia which can be reversed by treatments which restore serotonin content and presumably

serotonin function.

Analgesia has been observed following other drug treatments that increase the probability of serotonergic neurotransmission. For example, fluoxetine, a serotonin reuptake blocker, produces analgesia in rats presented with different intensities of electric footshock and antagonizes the hyperalgesia associated with PCPA treatment (Messing et al., 1975). Along similar lines, animals treated with quipazine, a serotonin receptor stimulator, also show analgesic responses as determined by the hot-plate method or tail compression test (Samanin et al., 1976). Moreover, the quipazine-induced analgesia can be antagonized by pretreatment with a serotonin receptor blocker (Samanin et al., 1976).

Similar to the findings observed with pharmacologic manipulations, electrical or chemical destruction of serotonergic neurons produced increased pain sensitivity. Harvey and co-workers (Harvey et al., 1974) found that electrolytic lesions of serotonin raphe nuclei which produced large reductions in forebrain serotonin concentration were correlated with increased pain sensitivity which could be reversed with 5-hydroxytryptophan (5-HTP; a serotonin biosynthetic precursor) but not with other amino acids not involved in serotonergic neurotransmission. It is presumed that serotonin is converted to serotonin in undamaged serotonin neurons which increase their neural activity to compensate for the lost serotonergic function. This finding lends support to the hypothesis that serotonin neurons are the critical factor involved in the development of hyperalgesia. Not all findings, however, are consistent with the hypothesis that a reduction in serotonin function is associated with an increase in pain sensitivity. Others have shown that electrolytic or chemical lesions of serotonin raphe nuclei produce no change

in pain responsiveness, whereas lesioning of serotonin axons or ter-
minal regions produce hyperalgesia. Hence, different lesions aimed at
serotonin cell bodies, axons or terminals seen to produce comparable
forebrain serotonin reductions, but only axon and terminal lesions
result in hyperalgesia. The reasons for these disparities are not
clear, but it has been suggested that destruction of serotonin cell
bodies may result in damage to adjacent hindbrain tissue (the central
gray area) which when damaged is known to produce analgesia. In ad-
dition, it is possible that the analgesia associated with serotonin
neurons is a post-synaptic receptor phenomenon which is abolished
following serotonergic nerve terminal destruction.

Although the results of Harvey et al. (1974) suggest that an
ascending serotonergic brain system is involved in mediating the be-
havioral response to noxious stimuli, data obtained from studies where
raphe nuclei have been stimulated electrically suggest that a descend-
ing serotonergic system (with cell bodies localized in the posterior
raphe nuclei and axons projecting caudally in the spinal cord) is also
important for pain sensitivity and responsivity (see Fig 3 for seroto-
nin neuroanatomical pathways). Electrical stimulation of the nucleus
raphe dorsalis, which projects rostrally, or the nucleus raphe magnus
which projects to the spinal cord, both produce analgesia in rats
(Samanin and Valzelli, 1971; Proudfit and Anderson, 1975). Interest-
ingly, the stimulation-produced analgesia elicited from the raphe
nuclei is enhanced in animals treated with serotonin (Akil and Liebes-
kind, 1975) and is blocked in animals pretreated with PCPA (Akil and
Mayer, 1973). Furthermore, lesions of spinal cord dorsolateral funicu-
lus, through which descending serotonin-containing neurons project,

Figure 3. A schematic diagram illustrating the distribution of the main serotonin pathways in the rat central nervous system. (Modified after G. Breese, Handbook of Psychopharmacology, Vol I, 1975). The nine clusters of raphe serotonin cell bodies are designated B1-B9 according to the nomenclature proposed by Dahlstrom and Fuxe (1964). The nuclear equivalents of these symbols are as follows: B1 - N. Raphe Pallidus; B2 - N. Raphe Obscurus; B3 - N. Raphe Magnus; B4 - small cell group close to N. Vestibularis Medialis; B5 - N. Raphe Pontis; B6 - Periventricular Gray region; B7 - N. Raphe Dorsalis; B8 - N. Raphe Medianus, and B9 - N. Raphe Centralis Superior
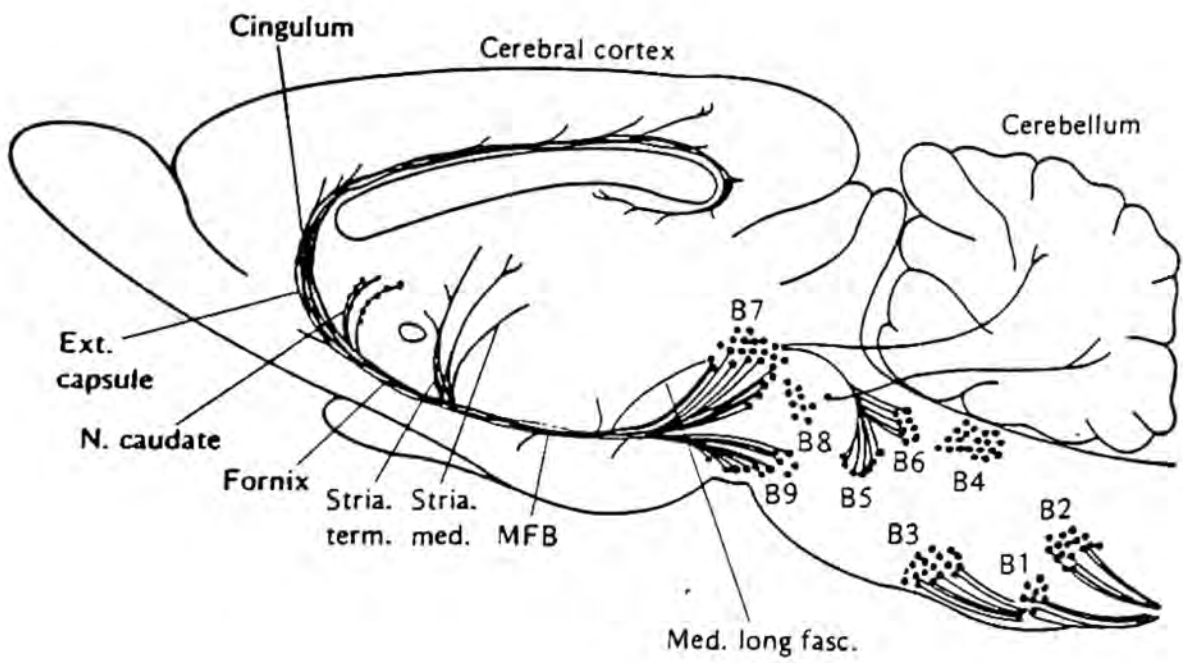
Figure 3

prevent stimulation-produced analgesia (Basbaum et al., 1977). Other
more indirect evidence also supports this hypothesis. Microiontopho-
retic applications of serotonin in the spinal cord inhibit the normal
electrical responses of dorsal horn spinal cord neurons to noxious
stimuli (Randic and Yu, 1976). More recently it has been shown that
local (intrathecal) injection of serotonin into the spinal cord of
mice produces analgesia and an inhibition of the spinal action of
intrathecally administered substance P (the neurotransmitter likely to
be involved in pain transmission between the primary afferent neuron
and ascending spinal cord neurons) (Hylden and Wilcox, 1983). Anatom-
ically, nucleus raphe magnus (NRM) serotonin neurons project to spinal
cord areas that receive primary afferent sensory input from somatic or
visceral nerves. NRM electrical stimulation results in strong inhibi-
tion of neurons in lamina I (marginal layer) and lamina V of the spinal
cord both of which respond to noxious inputs. In contrast, dorsal
horn neurons which respond maximally to innocuous mechanical stimula-
tion are not inhibited by NRM stimulation. In summary, anatomically
and physiologically, serotonin in the NRM constitutes a descending
inhibitory influence on pain transmission at the level of the spinal
cord dorsal horn and appears by this mechanism to be involved in stimu-
lation-produced analgesia as well.

Changes in nociception have also been observed following dietary
manipulations of serotonergic neurons. The rate at which serotonin is
synthesized in brain neurons is dependent, in part, on the availability
of its amino acid precursor, tryptophan. Tryptophan hydroxylase, which
hydroxylates tryptophan to 5-hydroxytryptophan, is not saturated at
normal brain concentrations of tryptophan, and it is also not regulated

by end-product inhibition. Increases in dietary amounts of tryptophan, therefore, result in increased serotonin synthesis (Fernstrom and Wurtman, 1971) and presumably increased serotonergic neurotransmission.

The results of recent experiments show that diet-induced alterations in serotonin synthesis are correlated with changes in pain responses that are similar to those seen after pharmacological, surgical or electrophysiological manipulations of serotonin. In one study, animals fed a low tryptophan diet and having reduced brain concentrations of tryptophan and serotonin were hyperalgesic to electric footshock. Furthermore, these nutrition-related effects could be reversed by adding tryptophan to the diet (Lytle et al., 1975). In other related experiments it was shown that PCPA treatment which depletes serotonin by inhibiting its synthesis potentiated the hyperalgesia produced following the administration of a tryptophan deficient diet (Messing et al., 1976). Furthermore, fluoxetine which increases synaptic concentrations of serotonin by blocking its reuptake into presynaptic neuron terminals, produced an analgesia to electric footshock in control and tryptophan-deprived animals.

In summary, pharmacological, physiological, surgical and dietary manipulations which presumably increase serotonergic neurotransmission are associated with a behavioral analgesia. Reductions in serotonergic neurotransmission, on the other hand, produce a hyperalgesia which under most circumstances can be reversed by restoring serotonergic function. However, the precise relationship that may exist between serotonin neurotransmission, endogenous opiate peptides and analgesia remains to be elucidated.

A variety of studies provide evidence that serotonin neurons are

important mediators of morphine analgesia. Manipulations which increase serotonergic neurotransmission enhance morphine analgesia (opiate analgesia), whereas manipulations which decrease it attenuate morphine's antinociceptive properties. Tenen (1968) was the first to show that PCPA-induced inhibition of brain serotonin synthesis antagonized morphine analgesia and that these effects could be reversed by serotonin administration. These basic findings have been reported in some, but not all, laboratories. Other studies using different drug manipulations of serotonin offer strong support for the neurotransmitter's role in analgesic drug potency. p-Chloroamphetamine and fenfluramine which have a biphasic effect on serotonin neurons-initially releasing serotonin and then reducing serotonin concentrations in brain and spinal cord – produce biphasic effects on the antinociceptive properties of morphine -initially enhancing morphine analgesia, later attenuating its effects (Duncan and Spencer, 1973). The neurotoxic drug 5,6-dihydroxytryptamine also attenuates morphine analgesia, apparently by destroying descending serotonergic neurons in the spinal cord (Vogt, 1974). Consistant with these findings, other serotonin drugs of known actions produce predictable changes in morphine analgesia. For example, morphine analgesia is enhanced in animals pretreated with the serotonin reuptake blocker, fluoxetine (Messing et al., 1975), or with intraventricular injections of serotonin (Sewell and Spencer, 1974) and is diminished in animals pretreated with serotonin receptor antagonists (Yaksh et al, 1976).

Neurophysiological and lesion studies further support the hypothesis that ascending and descending serotonergic raphe nuclei are important mediators of opiate analgesia. Electrical stimulation of ascending (N. Raphe Dorsalis and Medianus) and descending NRM raphe

nuclei potentiate morphine's analgesic potency (Samanin and Valzelli, 1971; Sharpe et al., 1974; Malick and Goldstein, 1976), whereas electrolytic or chemical lesions of these raphe nuclei attenuate opiate analgesia (Saminin et al., 1973; Proudfit and Anderson, 1975; Vogt, 1974). In addition, morphine administration has been shown to increase neuronal activity in the NRM (Oleson and Liebeskind, 1975) which appears to mediate a direct inhibitory effect on dorsal horn neurons involved in noxious input responses.

In summary, a rather wide variety of evidence based on studies utilizing pharmacological, electrophysiological, surgical or dietary manipulations indicates that brain and spinal cord cells utilizing serotonin as their neurotransmitter are intimately involved in nociceptive responses as well as in mediating SPA and some of the effects of opiate analgesia. In general, increases in serotonergic neurotransmission are associated with a decrease in pain sensitivity and/or reactivity and increases in analgesic drug potency. In contrast, decreases in serotonergic neurotransmission are associated with hyperalgesia and diminished opiate drug potency. It should be noted that other neurotransmitter systems are also known to interact in the brain and spinal cord to mediate normal and drug-induced changes in pain response. Taken together there is much evidence to suggest that SPA and opiate analgesia share common anatomical substrates involving the ascending and descending serotonergic pathways in the CNS.

Serotonin Neurons and Analgesia Involving Endogenous Opiates:

Evidence to date suggests that SPA and opiate analgesia share, at least in part, a common neurohumoral substrate - possibly the endogenous opiates. Many common features exist between the anatomy, physiology and pharmacology of SPA and opiate analgesia. The most effective sites for SPA and opiate analgesia by intracerebral injection overlap considerably. The ventrolateral PAG is the most effective site for both and there is cross-tolerance between opiate analgesia and SPA (Mayer and Hayes, 1975). The PAG is also rich in opiate receptors and endogenous opiate content. Finally, and most importantly, it has been shown that naloxone, a specific narcotic antagonist, partially blocks SPA (Akil et al., 1976). A most parsimonious explanation for the striking similarities between SPA and opiate analgesia is that brain stimulation causes the release of an endogenous opiate substance - the endorphins. In support of this hypothesis, CNS endorphin distribution correspond well to sites where opiate analgesia and SPA exert their analgesic effect and to the localization of opiate receptors.

There is also much evidence to suggest a functional link between the endorphins and serotonergic pathways mediating analgesia. Stimulation of raphe nuclei leads to analgesia that can be completely blocked by prior treatment with naloxone (Oliveras et al., 1977). Opiate analgesia requires functionally intact ascending and descending serotonergic pathways. The remarkable overlap between the regional distribution of spinal cord opiate receptors, enkephalins and the location of axon terminals from the NRM suggest that this raphe nucleus is involved in pain-suppression at the spinal cord level. Furthermore, large midbrain inputs to the NRM, including a projection from the ven-

trolateral PAG, have been described (see Basbaum and Fields, 1978).
This latter connection may mediate the analgesia produced by micro-
injection of opiates or electrical stimulation of the PAG. Other
anatomical and physiological data suggest that in addition to inputs
from opiate-sensitive brainstem structures, the NRM may also receive
major, though indirect, somatosensory input from pain-transmitting
systems (see Basbaum and Feilds, 1978).

The preceeding evidence has prompted Basbaum and Fields (1978)
to propose a model of the endogenous pain control system which may be
summarized as follows. Cells in the PAG and its rostral diencephalic
extention, the periventricular gray, can be activated by electrical
stimulation, opiate administration or possibly endogenous opiate release
at these sites. PAG cells, in turn, project to and activate cells in
the NRM and adjacent N. Reticularis gigantocellularis. These medullary
neurons project to the spinal dorsal horn where they inhibit pain
transmitting neurons which contain substance P as their neurotransmitter.
In the case of the NRM, an interposed enkephalinergic interneuron may
mediate serotonergic inhibition of pain transmission at the spinal cord
level. The pain-transmitting neurons (containing primary afferents)
project to supraspinal levels and indirectly, via the N. Reticularis
gigantocellularis, contact the cells of the descending analgesia system
in the PAG and NRM thus establishing a negative-feedback loop. Acti-
vation of the primary afferent pain-transmitting neurons by noxious
stimulation turns on the descending pain-suppression system which
effectively blocks further pain transmission at the spinal cord level.

It should be made clear that Basbaum and Field's model may des-
cribe only in part the total endogenous pain control system. The

model incorporates most of what is known about the neural circuitry of pain and the involvement of descending serotonergic pathways mediating pain suppression. Other neurotransmitter systems are also known to be involved in pain and analgesia. Much evidence to date suggests that CNS and circulating β-endorphins play an important role in pain and analgesia, and it is possible that endogenous opiates function in the circuit diagram of Basbaum and Fields and in ways not incorporated by their model. For example, the release of β-endorphin within limbic structures may serve to alter the perception of pain, and circulating β-endorphin which has been shown to desensitize peripheral pain receptors may provide another important component of endogenous pain control.

Interestingly, evidence suggests that the ascending serotonergic pathways may be important in regulating the release of pituitary β-endorphin and thus support pituitary β-endorphin involvement in serotonin-mediated analgesia. Anatomically, the N. Raphe Dorsalis and Medianus which provide the greatest innervation to hypothalamic regions, especially the median eminence (Descarries and Beaudet, 1978), are most likely involved in pituitary β-endorphin regulation since these neurons have been correlated with hypothalamic and anterior pituitary hormone release (see Weiner and Ganong, 1978). Many and varied neurophysiological and pharmacological studies have supported a stimulatory role of brain serotonin neurons in the regulation of CRF-mediated ACTH release (see Fuller, 1981), an important finding since pituitary ACTH and β-endorphin share common regulatory mechanisms. Some studies have shown that serotonin neurons have no influence or inhibit the release of pituitary ACTH (see Fuxe et al., 1974). The apparent disparity between these

reported results may be due to differences in methodology and specificity of the pharmacologic agents used since more recent studies consistently indicate a stimulatory role for serotonin neurons in ACTH release. It is also possible that serotonergic inhibitory/stimulatory control over ACTH may reflect the different post-synaptic events associated with the recently defined S_1 and S_2 serotonin receptor subtypes (see Peroutka and Snyder, 1981). Despite these discreptancies, in vitro studies have shown that serotonin can increase the content and release of CRF, a potent ACTH and β-endorphin releasing factor, from hypothalamic tissue (Buckingham and Hodges, 1970). More recently, Bruni et al. (1982) have shown that serotonergic drugs elevate plasma levels of β-endorphin-like immunoreactivity and ACTH in rats. Their results also indicated that serotonin neurons are involved, at least in part, in the stress-induced release of these peptides. These observations are consonant with the hypothesis that the analgesia associated with serotonin activation may be mediated through pituitary β-endorphin.

Other evidence suggesting a functional link between brain serotonin neurons and pituitary β-endorphin comes from stress studies. Various forms of physical stress known to increase the release of pituitary β-endorphin have been shown to enhance whole brain and hypothalamic serotonin metabolism (Mueller et al., 1976; Bliss et al., 1972; Palkovits et al., 1976), suggesting increased activity of serotonin neurons during stress. It has also been observed that under conditions of stress (immobilization, electric footshock, cold water swim) a behavioral analgesia occur in laboratory animals (Amir and Amit, 1978; Kulkarni, 1980; Grau et al., 1981). Moreover, this stress-induced analgesia can be partially or completely blocked by naloxone (Grau et al., 1981; Amir and Amit,

1978; Kulkarni, 1980), serotonin antagonists (Kulkarni, 1980; Bhatta-
charya et al., 1978; Lin et al., 1980) and hypophysectomy (Lewis et
al, 1980; Amir and Amit, 1978b; Bodnar et al., 1979). Together these
observations suggest that the behavioral analgesia that results as
a consequence of stress requires serotonergic neurotransmission and
opiate receptor ligands, which arise in part from a pituitary origin.
In addition, pharmacologic, neurophysiologic and dietary approaches
which increase serotonin neurotransmission result in a behavioral
analgesia which can be partially blocked by naloxone, further suggesting
a functional relationship between serotonin neurons and endogenous
opiates.

In summary, much evidence has accumulated to support the invol-
vement of serotonin neurons in endogenous mechanisms of analgesia.
Endogenous opiate peptides, of which β-endorphin is the most potent,
are also known to play an important role in pain and analgesia. Inter-
estingly, there appears to exist a functional relationship between
brain serotonin neurons and CNS and pituitary opiate peptides. Pontine
and medullary serotonin neurons projecting down the spinal cord modu-
late pain transmission probably through a mechanism which involves the
release of enkephalins from nerve terminals in the dorsal horn. In
addition, neural communication between serotonin neurons of the mid-
brain raphe nuclei and the PAG, a region rich in endorphinergic nerve
terminals, provides a second functional substrate of the endogenous
analgesia system. Indirect evidence suggests a role for serotonin neur-
ons in the regulation of pituitary β-endorphin which may represent a
third process involved in the development of analgesia. An important
objective of the present research was to better understand and define

the CNS mechanisms regulating pituitary-endocrine function in mammals, namely the regulation of β-endorphin, a potent analgesic peptide found in highest concentration in the pituitary gland. Further, it was hoped that an understanding of regulatory mechanisms governing pituitary β-endorphin release may provide insight into the possible physiological functions of pituitary and blood-borne β-endorphin peptides. Since β-endorphin$_{1-31}$ is only secreted in appreciable amounts by the pars distalis and is the only pituitary peptide with significant analgesic properties, it was important to determine the molecular forms and thus the pituitary source (anterior vs intermediate lobe) for β-endorphin-like immunoreactivity released into blood in response to serotonergic drugs and under different physiologic conditions. Together, these findings may further substantiate a functional relationship between brain serotonin neurons and pituitary β-endorphin release and hence provide a better understanding of the endogenous analgesia systems involving serotonin neurons and opiate peptides.

MATERIALS AND METHODS

A. Animals:

All experiments were conducted on mature, male, Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing 200-350 g. Animals were housed in groups of four at 22°C (lights on from 0600-1800 hr) and received standard rat food (Rat, Mouse, Hamster Formula, Charles River, Syracuse,NY) and tap water ad libitum.

All animals were accustomed to daily handling for five days and were acclimated overnight to the experimental room prior to the experimental day to minimize the effects of non-specific stress that might influence pituitary β-END release.

B. Treatments:

1. Drugs

The serotonergic drugs listed in Table 1 were administered as single intraperitoneal injections in a volume of 0.2 mg/100 g body weight, with the exception of 5-hydroxytryptamine hydrochloride and 5,7-dihydroxy-tryptamine creatinine sulfate which were injected in a volume of 3 µl into the lateral cerebral ventricle under ether anesthesia according to the method of Noble et al. (1967). The pharmacologic actions of the drugs listed in Table 1 have been reviewed by Fuller (1980). Drug doses and treatment times are indicated in Table 1, and animals receiving appropriate vehicle injections served as controls.

All experiments were conducted between 0800-1300 with the exception of the neurophysiologic studies which were conducted in the morning and afternoon. All treatments were administered in a randomized

Table 1. Serotonergic and Other drugs

Drug	Description of Action	Dose-Range Time Course	Distributor	Vehicle
L-tryptophan	Amino Acid serotonin precursor	75-200 mg/kg;ip 60 min	Sigma Chemical Co St. Louis, MO	1% carboxy-methylcel-lulose in 0.9% NaCl
D,L-5-Hydroxy-tryptophan (5-HTP)	immediate serotonin precursor	30 mg/kg;ip 30 min	Sigma Chemical Co St. Louis, MO	1% carboxy-methylcel-lulose in 0.9% NaCl
Quipazine maleate	serotonin receptor agonist	2.5-10 mg/kg;ip 15-90 min	Miles Laboratories Inc. Elkhart, IN	0.9% NaCl
5-Hydroxy-tryptamine HCl	serotonin	50ng-5µg;icv 15 min	Sigma Chemical Co St. Louis, MO	1% ascorbic acid in 0.9% NaCl
Fluoxetine HCl	serotonin reup-take blocker	10 mg/kg;ip 15 min	Eli Lilly & Co, Indianapolis, IN	0.9% NaCl
Cyproheptadine HCl	serotonin receptor antagonist	0.1-3.0 mg/kg;ip 60 min	Merck, Sharp & Dohme, West Point, Pennsylvania	0.9% NaCl
Cinanserin HCl	serotonin receptor antagonist	3-30 mg/kg;ip 60 min	Squibb Institute for Medical Res. California	0.9% NaCl

Drug	Description of Action	Dose-Range Time Course	Distributor	Vehicle
Pargylene HCl	monoamine oxidase inhibitor	25-225 mg/kg;ip 20-60 min	Regis Chemical Co Chicago, IL	0.9% NaCl
5,7-Dihydro-xytryptamine creatinine sulfate	serotonin neurotoxin	75-200 μg;icv 5-10 days	Regis Chemical Co Chicago, IL	1% ascorbic acid in 0.9% NaCl
Desipramine HCl	noradrenergic reuptake blocker	30 mg/kg;ip 30 min	USV Pharmaceuti-cal Corp, Tucka-hoe, NY	0.9% NaCl
Dexamethadone Sodium phos-phate	synthetic glucocorticoid	50 μg/kg;ip 4 hr	Carter-Glogau Labs, Glendale, AZ	0.9% NaCl
Pentabarbital	Anesthetic (general)	50 mg/kg;ip 20 min	Sigma Chemical Co St. Louis, MO	0.9% NaCl
Urethane-α-chloralose solution	Anesthetic (general)	1.3 g/kg - 95 mg/kg;ip, respectively	Sigma Chemical Co St. Louis, MO	0.9% NaCl

block design with 6-8 rats per treatment group.

2. Stress

Physical immobilization or etherization were used as near maximal
and submaximal stimuli for pituitary β-END-LI release, respectively
(see below and Mueller, 1981). Physical immobilization was administered
by loosely taping rats to test tube racks with masking tape and then
placing them on their backs. Thirty minutes later animals were freed
and decapitated to collect blood samples. Ether stress was administered
by placing rats in a saturated ether chamber for 90 seconds. Animals
were then allowed to recover in separate cages for 13 1/2 minutes.
Blood samples were collected by decapitation at 15 minutes after the
onset of etherization. Both stress procedures provide reproducible
stimuli for the release of pituitary β-END-LI without physically in-
juring the animals (Mueller, 1981). It is important to note that under
these conditions, etherization is approximately one half as potent in
stimulating β-END release as immobilization. Therefore, etherization
provides a submaximal stimulus for β-END release which can be either
facilitated or antagonized pharmacologically. By contrast, physical
immobilization, which results in far greater release of β-END, provides
a model for examining mechanisms which result in the inhibition of
β-END release.

C. Sample Collection:

1. Plasma samples

Trunk blood samples were collected by decapitation (within 15
seconds after removal from cage) into plastic tubes containing 0.6

ml 10% EDTA in 0.05M phosphate buffer (pH 7.3) and 50 mg% bacitracin (Sigma Chemical Co., St. Louis, MO). In the neurophysiologic studies, 0.5-1.0 ml of blood was collected over time from an indwelling femoral arterial catheter (PE-50 silastic tubing, Clay Adams Inc., Piscataway, NJ) into plastic tubes containing 0.1 ml 10% EDTA in 0.05M phosphate buffer (pH 7.3) and 50 mg% bacitracin. All blood samples were kept on ice until plasmas were separated by centrifugation (6,000 RPM, 20 min). Plasmas were stored in plastic tubes at -70°C until assayed for β-END-LI or processed further for gel filtration chromatography (see below).

2. Brain dissection and tissue collection

   a. Hypothalamus and whole brain collection

   For studies in which brain serotonin content was manipulated (serotonin precursors and neurotoxins) hypothalami or whole brains were rapidly removed (within 90 seconds of decapitation), dissected, frozen on dry ice and stored at -70°C until assayed for serotonin (within 24 hr). The hypothalamus was dissected as a region 3 mm in depth lying between the rostral borders of the optic chiasm and mammillary bodies and medial from the optic tracts. The average weight of hypothalamic tissue was 32±1 mg (n=32), and that of whole brain tissue was 1.7±0.01 g (n=48).

   b. Pituitary anterior and neurointermediate lobe dissection

   The anterior lobe (pars distalis) and neurointermediate lobe (pars intermedia plus pars nervosa; NIL) were dissected for tissue culture and chromatographic separation of β-END-LI peptides. Within 90 seconds

after decapitation, the brain was removed from the skull carefully to preserve the infundibulum until the pituitary stalk could be visualized and cut at the base of the brain. With the aid of a dissecting microscope and fine forceps, the NIL tissue was gently freed from its adhesions to the pars distalis. To avoid possible contamination of the pars distalis by cells of the pars intermedia, only the lateral poles of the pars distalis were collected.

D. Assays:

1. β-Endorphin radioimmunoassay

Immunoreactive β-endorphin-like peptides were measured using a radio-immunoassay (RIA) (Mueller, 1980) which is sensitive to less than 10 pg of camel β-endorphin$_{1-31}$ standard (Peninsula Labs, Inc., San Carlos, CA) The assay procedure is presented in Appendix II. Briefly, iodinated β-END was prepared by a standard chloramine-T reaction procedure (Greenwood et al., 1963) and purified on a SepPak™ C-18 reverse phase column (Waters Assoc., Milford, MA) using 40% acetonitrile/0.05% trifluoro-acetic acid as the eluent. The antibody [C-55; raised in a rabbit against camel β-END$_{1-31}$ conjugated to bovine thyroglobulin (Sigma)], used at a final dilution of 1:100,000, binds 30-35% of iodinated β-END in the absence of unlabelled peptide after 72 hrs. incubation at 4°C. The antibody detects purified human β-lipotropin (β-LPH) (gifts of Drs. A. Parlow and D. Orth) and the free and acetylated forms of camel β-END$_{1-27}$ and camel β-END$_{1-31}$ on an equimolar basis. The antibody does not recognize, however, the following peptides: α-END (β-END$_{1-16}$), γ-END (β-END$_{1-17}$), methionine enkephalin (β-END$_{1-5}$), leucine enkaphalin, α-melanocyte stimulating hormone (α-MSH), adrenocorticotropin (ACTH),

or other non-opiate hypothalamic or pituitary hormones. Dose-response curves of plasma, incubation medium, gel filtration fractions and pituitary and brain tissue extracts parallel camel β-END$_{1-31}$ standard. All samples of a given study were assayed in duplicate at two dilutions using the same RIA. In addition, plasma from hypophysectomized rats (Hormone Assay Labs, Chicago, IL) or heat-inactivated horse serum [Grand Island Biological Co. (GIBCO), Grand Island, NY] was included in the standard curve and non-plasma samples to control for non-specific plasma effects. Plasma levels of β-END-LI are expressed as ng/ml; chromatography elution profiles of plasma β-END-LI are expressed as ng/fraction and tissue levels of β-END-LI are expressed as ng β-END-LI/mg protein.

2. Serotonin Assay

Hypothalamic and whole brain concentrations of serotonin were measured by the spectrofluorometric method of Curzon and Green (1970) as modified by Hyypa et al. (1973). The details of this assay procedure are presented in Appendix III. Briefly, brain tissue was homogenized in acid butanol and centrifuged (17,000 RPM, 20 min). The supernatent was separated into an aqueous (0.1N HCl) and organic (heptane) phase. The aqueous layer (containing serotonin) was transferred into glass tubes containing 1% cysteine and then reacted with o-phthaldialdehyde solution at 100°C to induce fluorescence. Sample fluorescence was read at 355/480 nm in an Aminco-Bowman spectrophotofluorometer. Recoveries for serotonin standard extracted through this method averaged over 95%. Serotonin concentrations in tissue are expressed on a tissue weight basis.

3. Protein Assay

Protein content of brain tissues was measured using a Bio-Rad Protein Assay (Bio-Rad Labs, Richmond, CA) which is sensitive to 5 micrograms protein standard. Physiologically buffered saline solution (0.09% NaCl) was used to dilute protein standards (0.2 to 1.4 mg/ml), anterior lobe tissue (1:500) and neurointermediate lobe tissue (1:5,000). Diluted dye reagent (Bio-Rad Labs; 5 ml) was added to 0.1 ml aliquots of diluted protein standards and brain tissue samples and vortexed. After a period of 5 minutes to one hour, the optical density (OD_{595}) of each sample was read and compared to that of the standard curve (OD_{595} vs. micrograms protein standard concentration).

E. Separation of plasma β-END-LI peptides:

Gel filtration chromatography

Gel filtration chromatography was used to separate total β-END-LI on the basis of molecular size into two or three immunoreactive peaks co-eluting with β-LPH, $β-END_{1-31}$ standards and larger molecules eluting at the void volume. Pooled plasma samples (8 ml) from representative treatment groups were chromatographed on a Sephadex G-50 (fine or superfine resin, Pharmacia Inc., Piscataway, NJ) column (2.5 x 80 cm) and eluted (0.5 ml/min, 7.2 ml fractions) at 4°C with 0.1N acetic acid containing 0.05% bovine serum albumin, 0.02% sodium azide and 0.05 mg/ml bacitracin (chemicals from Sigma). Aliquots (2-3 ml) of each fraction were lyophilized and resuspended in (0.5-0.6 ml) assay buffer (0.05M phosphate solution containing 0.05% bovine serum albumin, 0.02% sodium azide and 0.05 mg/ml bacitracin; pH 7.3) and analyzed by RIA for β-END-LI. Recoveries for

β-LPH-sized and β-END-sized immunoreactivies were over 95%.

F. Determining the pituitary source (anterior vs. intermediate lobe) of
blood-borne β-END-LI:

In vitro, anterior lobe (AL) cells, but not intermediate lobe (IL)
cells, secrete a molecular form of β-END-LI which resembles β-LPH
standard (Fig. 12; Lissitsky et al. 1978, Smyth and Zakarian, 1980).
In addition, glucocorticoids selectively inhibit the release of β-END-LI
from cultured AL cells (Vale et al., 1979). It has also been shown that
the in vivo administration of glucocorticoids preferentially lowers plasma
levels of β-END-LI resembling β-LPH in molecular size (Mueller et al.,
1981) further suggesting the selective influence of glucocorticoids
over AL β-END-LI secretion. Thus the secretion of β-END-LI correspond-
ing to β-LPH and inhibition of this release by glucocorticoids provides
an approach for studying the physiologic regulation of pituitary β-END-LI
in vivo.

Plasma levels of β-END-LI resembling β-LPH and $\beta\text{-END}_{1-31}$ in molecular
size were resolved using gel filtration chromatography (as described above).
The distinctive elution profiles of immunoreactive β-END released by AL
and IL cells in vitro were compared to those of β-END-LI present in the
plasma of rats treated with serotonergic drugs. Changes in plasma levels
of β-END-LI resembling β-LPH served as a marker for β-END-LI release
from the AL in vivo.

To further examine serotonergic influence over AL and IL β-END-LI
secretion, the effects of dexamethasone on the molecular forms of β-END-LI
released in response to serotonergic stimulation were studied. Animals
were pretreated with 50 μg/kg (ip) dexamethasone sodium phosphate (Carter-

Glogua Labs, Glendale, AZ) for 4 hours prior to treatment with fluoxetine (10 mg/kg, ip, 15 min). The blood collected was subjected to gel filtration chromatography to examine the effects of dexamethasone treatment on plasma elution profiles. Since dexamethasone has been shown to selectively influence AL but not IL β-END-LI secretion, a reduction in the fluoxetine-induced rise of β-LPH-sized immunoreactivity following dexamethasone treatment would further substantiate serotonergic influence over AL β-END-LI release.

G. Pituitary Cell Culture:

Primary cell cultures of rat anterior lobe (AL) or neurointermediate lobe (NIL; pars intermedia plus pars nervosa) were established according to the method of Vale et al. (1978). Pituitary tissues were removed (see Materials and Methods, Sample Collection 2b) from 40 young male Sprague-Dawley rats weighing 100-150 g which were killed by decapitation. Tissues were immediately placed in an ice-chilled solution of Dulbecco's Modified Eagle's Medium containing 2% horse serum (DMEM; GIBCO).

Pooled NIL and AL tissues were minced and washed three time with Hank's Balance Solution and then centrifuged (2000 RPM, 10 min). Tissues were then dissociated enzymatically with a solution containing 3.5 mg/ml collagenase, 1 mg/ml hyaluronidase and 30 mg/ml albumin. The fluid layer was removed and tissues were again dissociated with pancreatin/Vio Kase (Sigma). Approximately $3x10^4$ NIL cells and $7x10^4$ AL cells were plated [10x16 mm and 15x35 mm culture dishes, respectively, (Falcon Plastic Co., Los Angeles, CA) in a culture medium consisting of DMEM (Hi-glucose), 10% horse serum, 2.5% fetal calf serum (GIBCO), 0.1 mM glutamine, and 1% non-essential amino acids (GIBCO). Cells were

grown at 37°C in an atmosphere of 95% air:5% CO_2.

After the cells had become established (7-9 days), the plates were washed four times with 2 ml DMEM containing 2% horse serum. The cells were then exposed to various test subtances (serotonin HCl or cortico-tropin releasing factor, CRF) in 2 ml of incubation medium (DMEM containing 2% horse serum plus 30 µg/ml bacitracin) for 90 minutes. Drugs were initially dissolved at a 1 mM concentration of 0.3 mM ascorbid acid (Sigma). Further dilutions were made in incubation medium making the final concentration of ascorbic acid 3 µM. This amount of ascorbid acid does not influence basal release of β-END-LI from AL or NIL cells (Pettibone and Mueller, 1982). After incubation, medium was withdrawn, centrifuged (2500 RPM, 20 min), decanted into plastic 12x75 mm test tubes (Columbia Diagnostics Inc., Springfield, VA) and frozen (-70°C) until assayed.

H. Neurophysiologic investigation of raphe nuclei:

The neurophysiologic studies were designed to further examine the role of two raphe serotonergic nuclei in the regulation of pituitary β-END secretion. Rats were anesthetized with a urethane (1.3 g/kg; Sigma)-α-chloralose (95 mg/kg; Sigma) solution and the right femoral artery was cannulated using PE-50 tubing (Clay Adams, Piscataway, NJ) for blood sampling. The rat was then placed in a stereotaxic apparatus (David Kopf Model 900 instrument, Tujunga, CA) and the cranium was exposed. A small burr hole was drilled (Teledyne Emesco, NJ; dental Bur no. 4) through the skull at the level of the Dorsal Raphe Nucleus (DRN) and Median Raphe Nucleus (MRN) (1.3-1.4 mm anterior to lambda and on the midline). The sagittal vein was punctured and bleeding was controlled using gelfoam

powder (Upjohn, Kalamazoo, MI). A stainless steel concentric bipolar
electrode (SNE-100, Rhodes Medical Instruments, 60 micron barrel diameter,
10 micron tip diameter) was lowered through the burr hole stereotaxically
and advanced to the level of the DRN (8.3 mm ventral from skull surface)
or the MRN (10.3 mm ventral from the skull surface). A biphasic (0.1-1.0
msec duration) constant current (0.05-0.5 mAmps) stimulus (10 pulses
per second) was delivered using a Grass Instruments S-88 stimulator and
Photoelectric Stimulus Isolation Unit with constant current output
(Grass Instruments, Quincy, MA). The current passed through the electrode
was monitored on a Techtronix 5115 oscilloscope (Tektronix Inc., Beaverton,
OR). Blood samples (0.5-1.0 ml) were withdrawn before, during and after
3-15 minutes of stimulation and equal volumes of 0.9% saline were reinfu-
sed after each blood withdrawal. Blood was collected into 1 cc plastic
tuberculin syringes containing 0.1 cc of 10% EDTA with 50 µg/ml baci-
tracin. Blood was transferred into 1.5 ml plastic microcentrifuge tubes
(AGonics, Brooklyn, NY) and stored on ice until centrifuged. Plasma was
stored at -70°C until assayed by RIA for β-END-LI. Control animals
were treated similarly with an electrode placed in the DRN or MRN but
no current was passed. Electrode placement in all animals was verified
by first marking the site by passing a direct current (4 µA, 20 sec.)
through the electrode tip and then perfusing the animal with a 10% for-
malin-phosphate buffer solution (Fisher Scientific Fairlawn, NJ) con-
taining 1% potassium ferricyanide. The potassium ferricyanide reacts
with the deposited iron from the electrode tip and results in a blue
stain which marks the site of the electrode tip. Brains were removed
and frozen for serial sectioning (36 micron slices) on a Damon-IEC
Refrigerated Microtome, Needham Hts., MA. Brain slices were mounted on

glass slides and observed under a light microscope.

I. Statistical Analysis:

Statistical analysis of data was carried out on a Hewlett Packard RT 3 computer using analysis of variance (one-way analysis of variance for dose-response and time course studies; two-way analysis of variance for combined treatment studies) followed by Duncan's Multiple Range Test for comparison (Winer, 1970). The level of significance was chosen to be $p < 0.05$.

THE EFFECTS OF SEROTONIN PRECURSORS ON PLASMA LEVELS OF β-END-LI

In order to understand how pharmacologic agents can be used to se-
lectively alter serotonergic neurotransmission, it is important to appre-
ciate what is known about the normal function of serotonin neurons (Fig.
4). Tryptophan is an essential amino acid which is actively transported
into serotonin neurons where it is hydroxylated to form 5-hydroxytryp-
tophan (5-HTP) by tryptophan hydroxylase. This enzyme controls the
rate-limiting step in the synthesis of serotonin but the precise manner
by which it is regulated is not completely understood. Tryptophan hydro-
xylase is not tightly regulated by an end-product inhibitory mechanism,
and it is not saturated by concentrations of tryptophan normally found
in the brain. Thus, changes in brain concentrations of tryptophan can
alter the rate of serotonin synthesis (Fernstrom & Wurtman, 1971).

Once synthesized, 5-HTP is almost immediately decarboxylated by
an aromatic amino acid decarboxylase to yield 5-hydroxytryptamine or
serotonin. (This decarboxylase enzyme is thought to be the same enzyme
involved in the biosynthesis of catecholamines.) Newly synthesized
serotonin, in turn, may be stored within intraneuronal vesicles or
released from the nerve terminal in response to a neuronal impulse,
electrical stimulation or drugs (Muller et al., 1977).

Following its release into the synaptic cleft, serotonin can bind
reversibly with either post-synaptic or pre-synaptic serotonin receptors.
Binding to post-synaptic receptors completes the process of neurotrans-
mission across the synapse. Pre-synaptic receptors located on serotonin
neurons themselves appear to function as autoreceptors modulating the
further synthesis and release of serotonin according to the concentration

Figure 4. A schematic of a serotonin neuron and how it functions. The amino acid tryptophan is taken up by the neuron and hydroxylated to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TOH). 5-HTP is subsequently decarboxylated to 5-hydroxytryptamine (5-HT; serotonin) by L-aromatic amino acid decarboxylase (L-AADC). Serotonin is stored in vesicles from which it is released into the synaptic cleft in response to nerve impulses. Within the cleft it may combine reversibly with post-synaptic receptors (action on these receptors completes the process of neurotransmission across the synapse) or pre-synaptic receptors (these receptors modulate the synthesis and release of serotonin). The action of serotonin on synaptic receptors is terminated by serotonergic reuptake into the nerve terminal where it may either be re-incorporated into storage granules or degraded enzymatically by a monoamine oxidase (MAO)/aldehyde dehydrogenase (ADH) pathway to 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite.
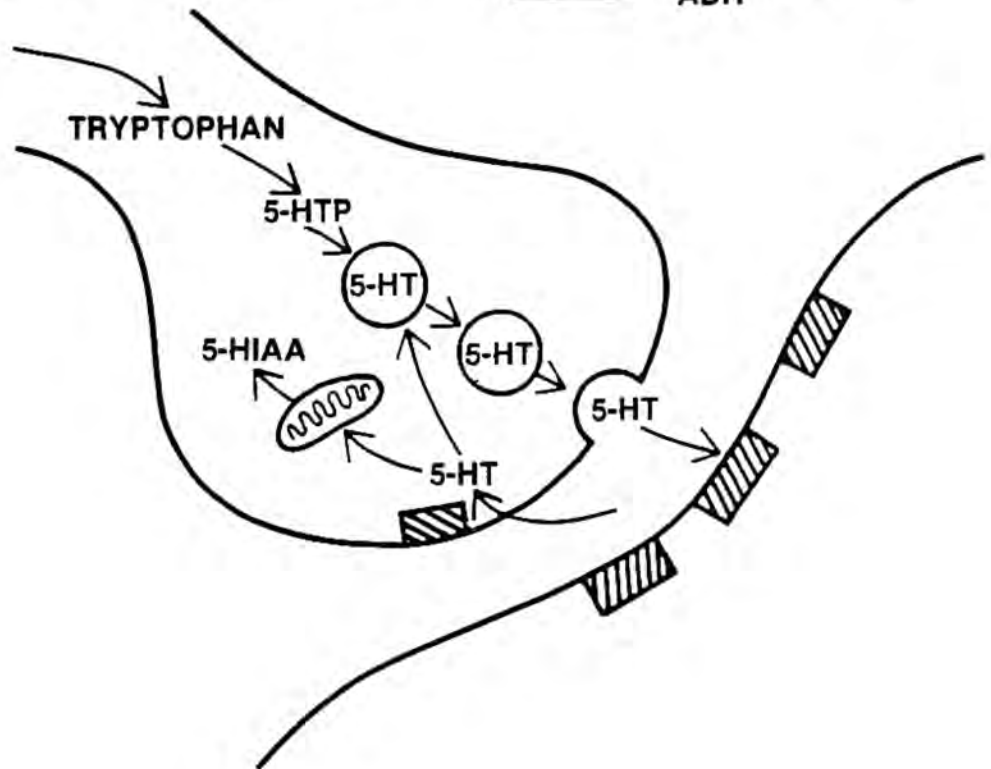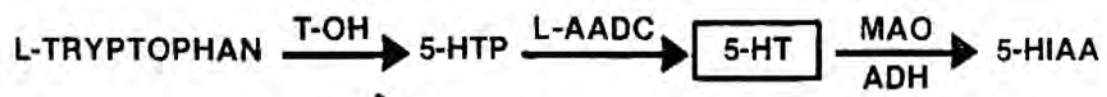
Figure 4

of serotonin in the synaptic cleft.

Serotonin's action at post- or pre-synaptic receptors is terminated when serotonin is either metabolized by extraneuronal monoamine oxidase (MAO) or is transported back into the nerve terminal by a stereospecific active uptake mechanism. Within the nerve terminal, serotonin may either be re-incorporated into storage vesicles or degraded enzymatically through a MAO pathway to 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite. Changes in the rates of serotonin synthesis and metabolism, as judged by changes in the ratio of serotonin/5-HIAA have been used as an index of serotonergic neuronal activity or turnover.

Recently, two distinct serotonin receptors, designated S_1 and S_2, have been differentiated in the central nervous system (CNS) on the basis of radioligand binding studies. S_1 receptors, which are selectively labeled by tritiated serotonin, appear to be coupled to adenylate cyclase and are regulated by guanine nucleotides (Peroutka et al., 1979). At this receptor site, agonists are generally more potent than antagonists at competing with the radioligand for binding. The S_2 receptors, which are labeled by tritiated spiperone, are not influenced by nucleotides. At this receptor, currently known antagonists generally are more potent than agonists at competing with the radioligand for binding.

Subsequent studies have provided evidence to suggest that S_1 and S_2 receptors mediate different physiologic functions (see Peroutka et al., 1981). By comparing drug affinities for S_1 and S_2 binding sites with drug potencies in blocking serotonin behavioral effects in rodents, it has been inferred that S_2 receptors mediate the "central serotonin syndrome". This behavioral hyperactivity which follows central serotonin stimulation is characterized by resting tremor, splayed hindlimbs,

side-to-side head weaving and head twitching. Accordingly, serotonin antagonists which block this serotonininduced behavioral syndrome bind S_2 receptor sites _in vitro_ with higher affinity than S_1 sites. Similar studies have shown that drug potencies as inhibitors of serotonin-sensitive adenylate cyclase correlate well with their affinities for S_1 but not S_2 receptors suggesting that S_1 receptors are regulated by guanine nucleotides, a phenomenon commonly reflecting a linkage to adenylate cyclase.

In addition to playing a role in numerous behavioral systems, serotonin is known to elicit both synaptic inhibition and excitation in the brain (Haigler & Aghajanian, 1974). Some data suggests that these differential neurophysiological actions of serotonin may also reflect discrete effects at S_1 or S_2 receptors. Synaptic excitation by iontophoretically applied serotonin is antagonized by drugs which are 40 to 400 times more potent at S_2 than S_1 receptors. Conversely, drugs which influence iontophoretic serotonin inhibition, are 15 to 200 times more potent at S_1 than S_2 receptors (see Peroutka et al., 1981). These data suggest that serotonin inhibition and excitation are mediated by S_1 and S_2 receptors, respectively.

In general, the relationship between S_1 receptors and both the serotonin-sensitive cyclase and neural inhibition is less clear than the link between S_2 sites and synaptic and behavioral excitation. A major problem in evaluating S_1 receptor binding and function is the lack of specific, high affinity antagonists for the S_1 receptor. In addition, in some cases there is no known correlation between functional responses and affinity for binding sites suggesting that other serotonin receptor types may be mediating these responses or other factors may prevent

demonstration of correlation (factors such as tissue distribution and metabolism of drugs in the case of in vivo studies).

The administration of serotonin precursors has been shown to increase brain serotonin synthesis (Grahame-Smith, 1971; Aghajanian & Asher, 1971) and hypothalamic serotonin metabolism (Mueller et al., 1976) suggesting increased serotonin neurotransmission. To determine whether serotonin neurons exert an influence over the basal release of pituitary β-endorphin (β-END), the effects of serotonin precursors on plasma levels of β-END-like immunoreactivity (β-END-LI) were studied. A single injection of 200 mg/kg (ip) L-tryptophan (TRYP) elevated brain serotonin content and doubled circulating levels of β-END-LI by 30 min (Table 2). The tendency for a smaller dose of TRYP (75 mg/kg for 30 min) to increase brain serotonin content was not significant, although plasma levels of β-END-LI were increased ($P<0.05$) in these animals. Treatment with 5-HTP (30 mg/kg, ip, for 30 min) produced a 4.9-fold increase in brain serotonin content and raised plasma β-END-LI levels from 0.67 ± 0.11 to 2.30 ± 0.20 ng/ml (Fig 5). The ability of 5-HTP to elevate brain serotonin content and plasma β-END-LI levels was considerably greater than that of TRYP, and it should be noted that this difference may be partially due to the less specific action of 5-HTP. Unlike TRYP which is hydroxylated only within serotonin neurons (Aghajanian & Asher, 1971), 5-HTP can be decarboxylated to serotonin in catecholamine neurons containing the aromatic amino acid decarboxylate (Ng et al., 1972). At high concentrations, 5-HTP can cause serotonin to displace endogenous catecholamines from catecholaminergic terminals following its uptake into these neurons and subsequent decarboxylation to serotonin. Since dopaminergic neurons can exert an inhibitory influence over the secretion

Table 2. Effects of tryptophan (TRYP) on brain serotonin content and

plasma levels of β-END-LI

Table 2

Group (n=8)	Brain serotonin (ng/g)	Plasma β-END-LI (ng/ml)
Control	456 ± 23	0.30 ± 0.06
TRYP (75 mg/kg)	536 ± 30	0.56 ± 0.07*
TRYP (200 mg/kg)	541 ± 29*	0.64 ± 0.08*

*Value significantly greater than vehicle-injected control value (P<0.05).

Figure 5. Effects of 5-hydroxytryptophan (5-HTP) on brain serotonin (5-HT) and plasma β-END-LI concentrations. Two groups of rats (n=8) were injected ip with either vehicle or 30 mg/kg 5-HTP 30 min before decapitation. Each bar and vertical line represents the mean ± standard error. Animals treated with 5-HTP had significantly higher (p<0.05) brain serotonin contents and plasma β-END-LI levels compared to those in vehicle-injected controls.
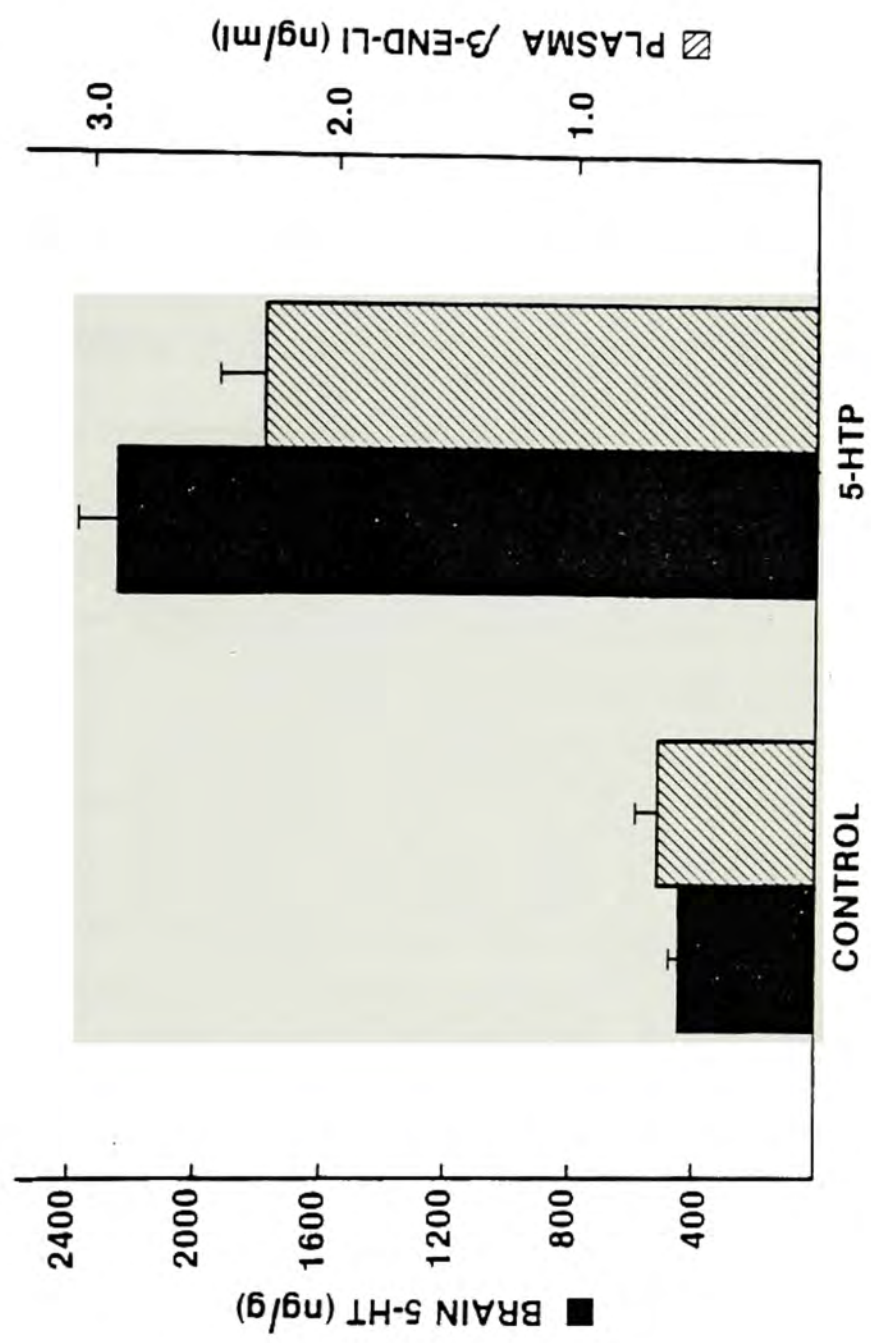
Figure 5

of β-END-LI in rats (Giraud et al., 1980; Farah et al., 1982), 5-HTP treatment may stimulate β-END-LI release through a combined action to enhance serotonin neurotransmission and inhibit dopaminergic neurotransmission.

In summary, serotonin precursors which increase brain serotonin content and presumably serotonergic neurotransmission, increase circulating levels of β-END-LI. These findings suggest that serotonin neurons exert a stimulatory influence over the secretion of pituitary β-END.

EFFECTS OF SEROTONERGIC DRUGS ON PLASMA LEVELS OF β-END-LI

To further investigate the role of serotonin neurons in the regulation of pituitary β-END secretion, the effects of several classes of serotonergic drugs on plasma β-END-LI levels were examined. Quipazine, a drug which mimics the action of serotonin at its post-synaptic receptor, at a dose of 5 mg/kg elevated plasma β-END-LI levels from 0.55 ± 0.07 to 1.17 ± 0.20 ng/ml by 15-45 min; by 90 min post-injection, circulating levels of β-END-LI had returned to control values (Fig 6). The administration of 2.5 and 5.0 mg/kg quipazine (ip, for 30 min) elevated resting levels of plasma β-END-LI in a dose-related manner (0.49 ± 0.06 vs. 1.26 ± 0.23 vs. 1.75 ± 0.08 ng/ml). A higher dose of quipazine (10 mg/kg, ip, for 30 min) had no further effect (Fig 6). Furthermore, the rise in plasma levels of β-END-LI in response to quipazine treatment was completely blocked by 60 min pretreatment with cinanserin (5 mg/kg, ip), a serotonin receptor antagonist (Fig 7). However, cinanserin treatment alone (3.0-30 mg/kg, ip, 60 min) had no significant effect on circulating levels of β-END-LI (Fig 8).

Fluoxetine (10 mg/kg, ip), a drug which inhibits serotonin reuptake

Figure 6. Time course and dose response effects of quipazine, a serotonin receptor agonist, on plasma levels of β-END-LI. Time course (top): Four groups of rats (n=8) were injected ip with either 5 mg/kg quipazine or vehicle. Trunk blood was collected 15, 45 and 90 min after quipazine injection and 15 min after vehicle injection. Each bar and vertical line represent the mean ± standard error. Quipazine-treated animals had significantly higher ($p < 0.05$) levels of β-END-LI 15 and 45 min but not 90 min post-injection. Dose response (bottom): Four groups of rats (n=7-8) were injected ip with either vehicle or 2.5, 5 or 10 mg/kg quipazine 30 min before decapitation. Compared to vehicle-injected control values, quipazine-treated animals (at all doses tested) had significantly elevated ($p < 0.05$) levels of circulating β-END-LI.
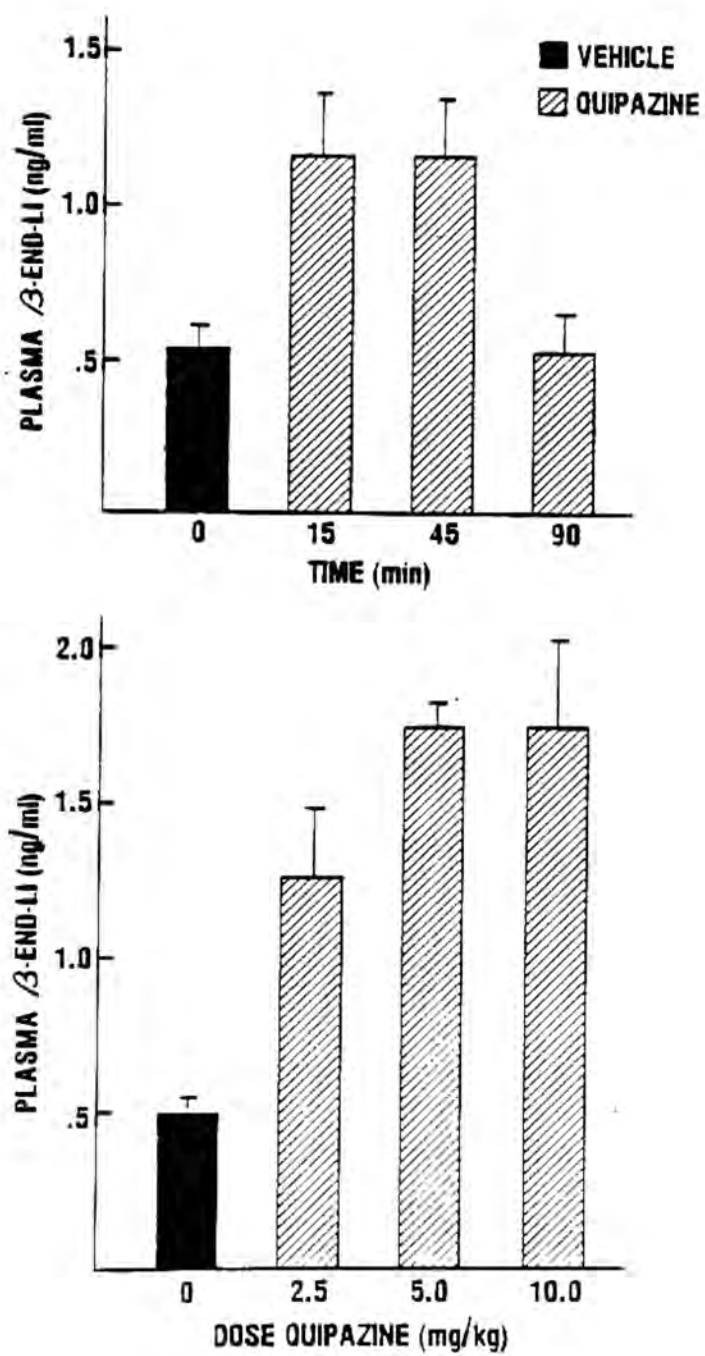
Figure 6

Figure 7. Effects of cinanserin, a serotonin blocker, on quipazine-stimulated levels of plasma β-END-LI. Four groups of rats (n=8) were pretreated (ip) for 60 min with either cinanserin (5 mg/kg) or its vehicle. Thirty min prior to blood collection, animals were injected ip with either quipazine (5 mg/kg) or its vehicle. Each bar and vertical line represent the mean ± standard error. Compared to control values, quipazine treatment significantly elevated (p<0.05) levels of circulating β-END-LI, whereas, cinanserin treatment alone had no effect on plasma β-END-LI levels. However, cinanserin pretreatment completely blocked the rise in plasma levels of β-END-LI in response to quipazine.
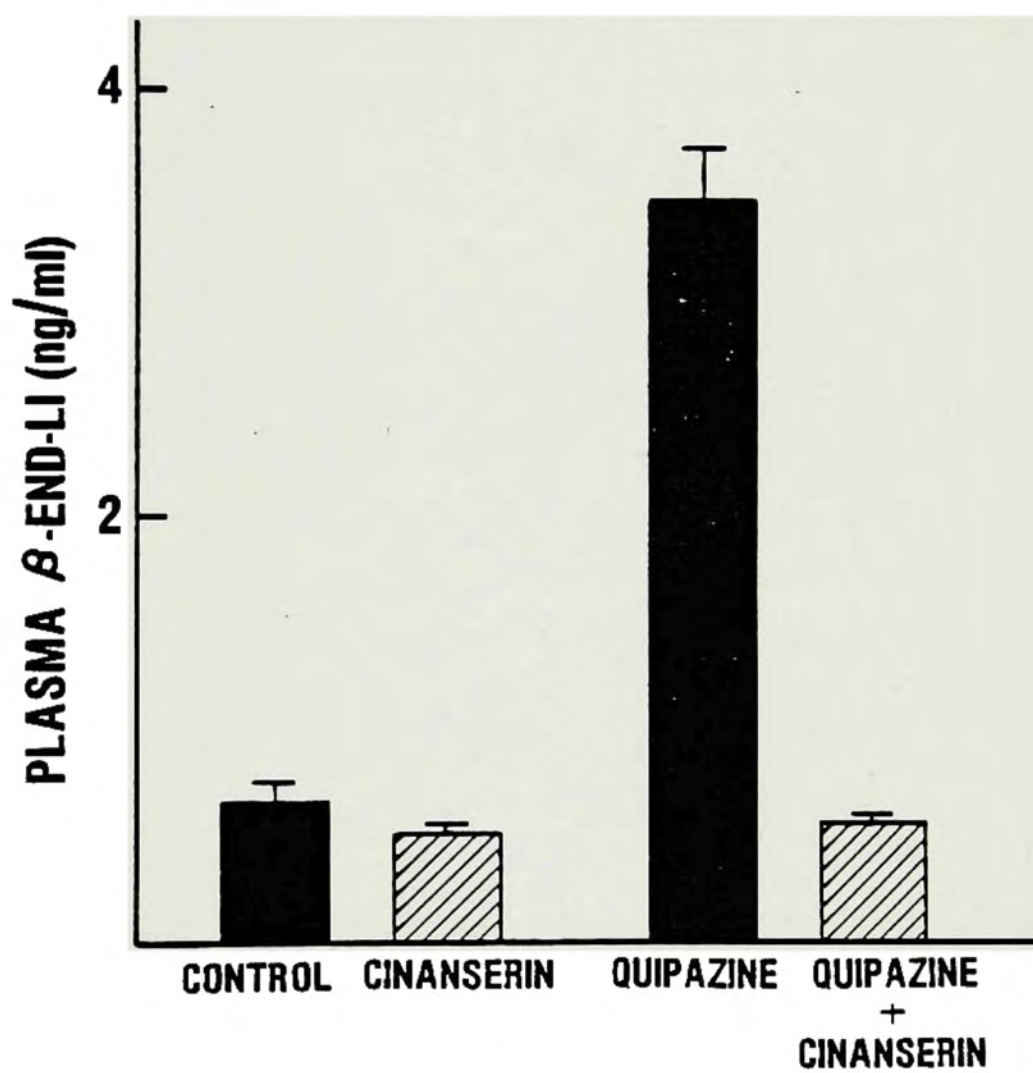
Figure 7

Figure 8. Time course and dose response effects of cinanserin, a serotonin receptor antagonist, on plasma β-END-LI levels. Time course (top): Four groups of rats (n=8) were injected ip with either 10 mg/kg cinanserin or vehicle. Trunk blood was collected 30, 60 and 120 min after cinanserin treatment and 15 min after vehicle injection. Each bar and vertical line represent the mean ± standard error. Plasma levels of β-END-LI in cinanserin-treated animals were not significantly different from vehicle-injected control animals at all times tested. Dose response (bottom): Four groups of rats (n=8) were injected ip with either vehicle or 3, 10, 30 mg/kg cinanserin 60 min prior to blood collection. Plasma levels of β-END-LI in cinanserin-treated animals were not significantly different from vehicle-injected control animals at all doses tested.
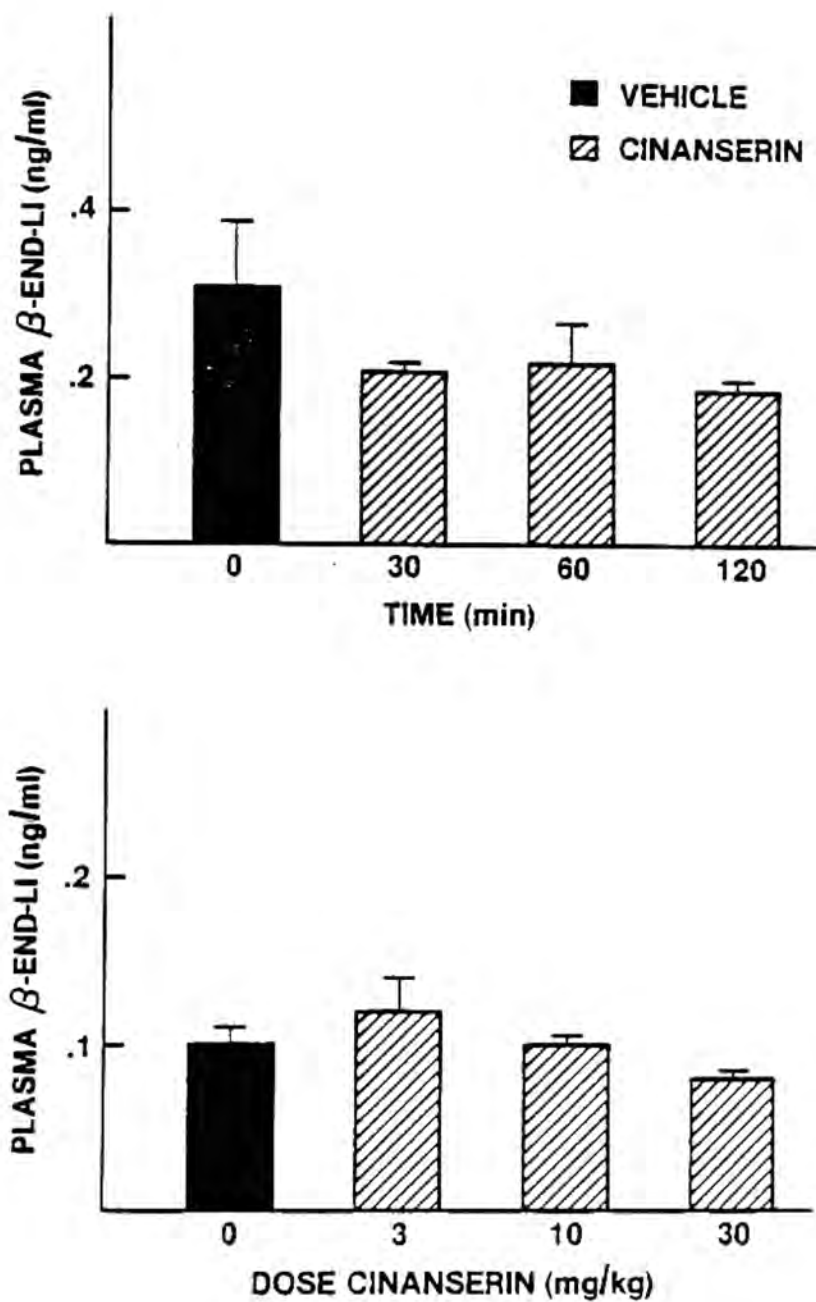
Figure 8

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Figure 9. Effects of increasing doses of cyproheptadine, a sero-
tonin receptor blocker, on fluoxetine-stimulated levels of plasma
β-END-LI. Four groups of rats (n=8) were pretreated (60 min, ip)
with either vehicle or 0.1, 1.0, or 3.0 mg/kg cyproheptadine. The
animals pretreated with 0, 0.1 or 1.0 mg/kg cyproheptadine were in-
jected ip with 10 mg/kg fluoxetine 15 min prior to blood collection.
A fifth group of animals (n=8) received double injections of vehicle
and served as the control group. Each bar and vertical line repre-
sent the mean ± standard error. Compared to the vehicle-injected
control values, fluoxetine treatment significantly elevated ($p<0.05$)
circulating levels of β-END-LI. Cyproheptadine at a dose of 0.1 and
1.0 mg/kg significantly attenuated ($p<0.05$) the plasma β-END-LI res-
ponse to fluoxetine treatment. However, a higher dose of cyprohep-
tadine (3.0 mg/kg) had no effect on resting levels of plasma β-END-LI
as compared to control levels of β-END-LI.
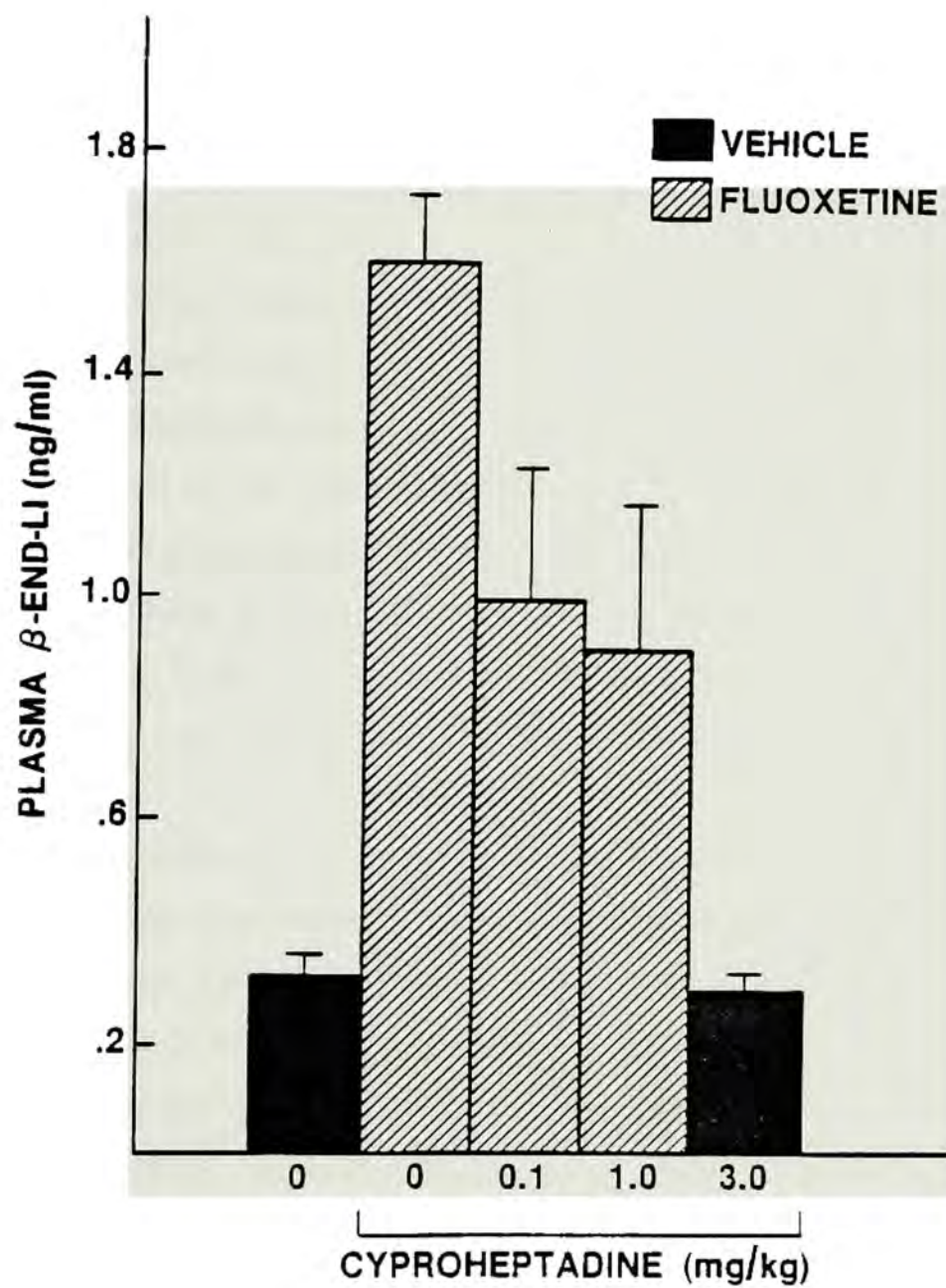
Figure 9

and therefore prolongs serotonin's activity within the synapse, elevated basal levels of plasma β-END-LI from a control value of 0.32 ± 0.04 to 1.61 ± 0.12 ng/ml by 15 min post-injection (Fig 9). Pretreatment (60 min) with a serotonin receptor blocker, cyproheptadine (0.1 or 1.0 mg/kg, ip) significantly attenuated the plasma β-END-LI response to fluoxetine treatment (Fig 9). Cyproheptadine pretreatment (3 mg/kg, ip, 60 min) alone did not alter resting levels of plasma β-END-LI as compared to vehicle-injected control values (Fig 9).

Since peripherally administered serotonin is rapidly degraded before significant amounts enter the central nervous system (CNS), serotonin was administered centrally. Intraventricular administration (icv) of 50 ng, 100 ng and 5 μg serotonin in pentabarbital-anesthetized rats tended to increase plasma levels of β-END-LI in a dose-related fashion. However, because of the large standard error within groups, only the highest dose (5 μg) was shown to significantly increase plasma levels of β-END-LI over control values by 15 min (90.0 ± 10 vs. 300.0 ± 75 pg/ml; Fig 10).

Inhibition of the enzyme that degrades serotonin (monoamine oxidase, MAO) has been shown to increase neuronal stores of serotonin (Tozer et al., 1960) which apparently results in increased concentrations of serotonin in the synaptic cleft due to the release of larger quantities of serotonin per nerve impulse. Thus the administration of pargylene, an MAO inhibitor, is another pharmacologic approach for enhancing serotonergic neurotransmission. However, it should be noted that pargylene does not selectively affect serotonin degradation since other biogenic amines are known to be degraded by MAO as well. Pargylene administration (75 mg/kg, ip) elevated plasma β-END-LI levels from 0.51 ± 0.05 ng/ml to

Figure 10. Dose response effects of intraventricular injections of serotonin on plasma levels of β-END-LI. Four groups (n=6-7) of pentabarbital-anesthetized rats (50 mg/kg, ip, 20 min) were injected intraventricularly with either vehicle or 50 ng, 100 ng or 5 µg serotonin. Fifteen minutes after injection, animals were decapitated and trunk blood collected. Each bar and vertical line represents the mean ± standard error. Compared to vehicle-injected control values, only the 5 µg dose of serotonin significantly elevated ($p < 0.05$) plasma levels of β-END-LI.
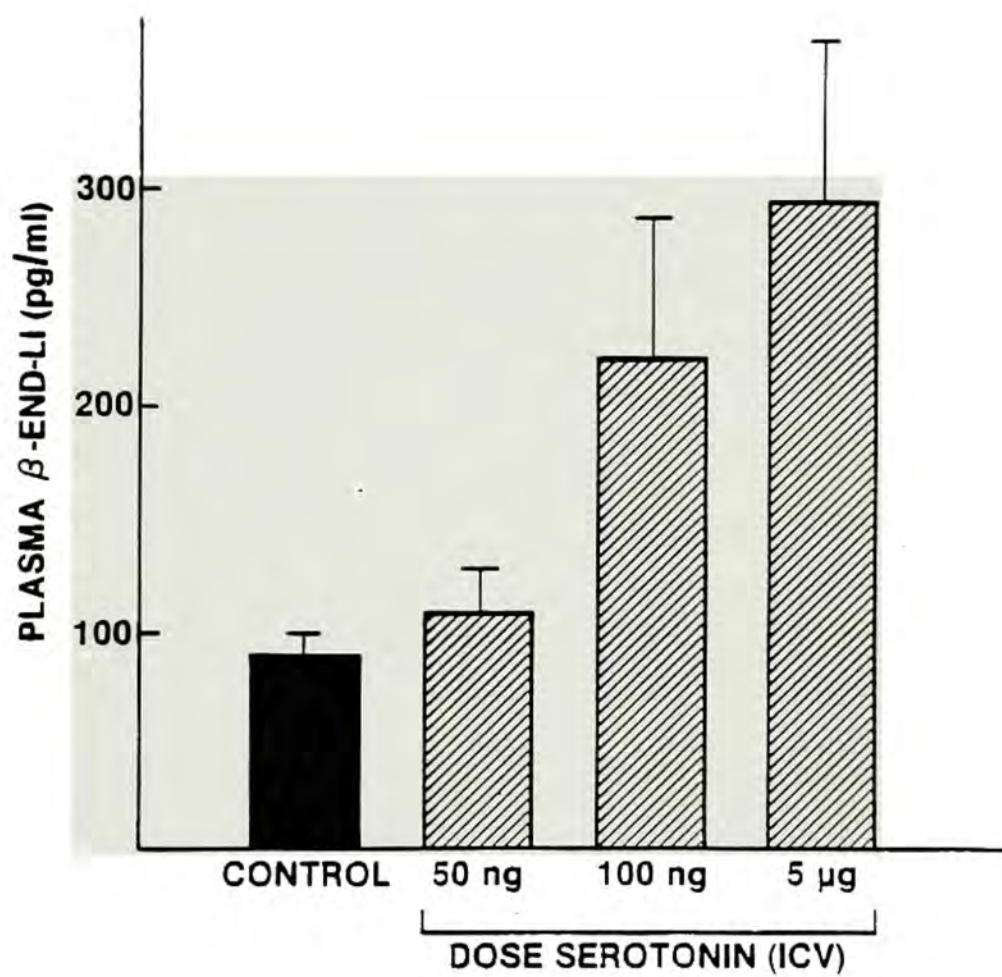
Figure 10

Figure 11. Time course and dose response effects of pargylene, a monoamine oxidase inhibitor, on plasma levels of β-END-LI. Time course (top): Four groups of rats (n=8) were injected ip with either vehicle (solid bar) or 75 mg/kg pargylene (hatched bars). Blood was collected 20, 40 and 60 min after pargylene injection and 15 min after vehicle injection. Each bar and vertical line represent the mean ± standard error. Compared to vehicle-injected control animals, pargylene-treated animals had significantly higher ($p < 0.05$) levels of β-END-LI at 20 and 40 but not 60 min post-injection. Dose response (bottom): Four groups of rats (n=8) were injected ip with either vehicle (solid bar) or 25, 75, or 225 mg/kg pargylene (hatched bars) 45 min prior to blood collection. Compared to vehicle-injected control values, 75 and 225 mg/kg pargylene significantly elevated ($p < 0.05$) plasma levels of β-END-LI.
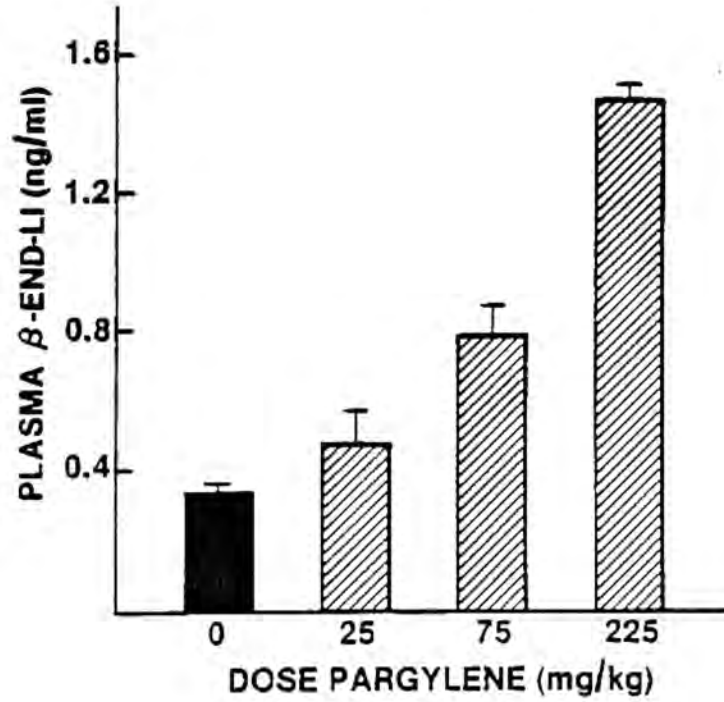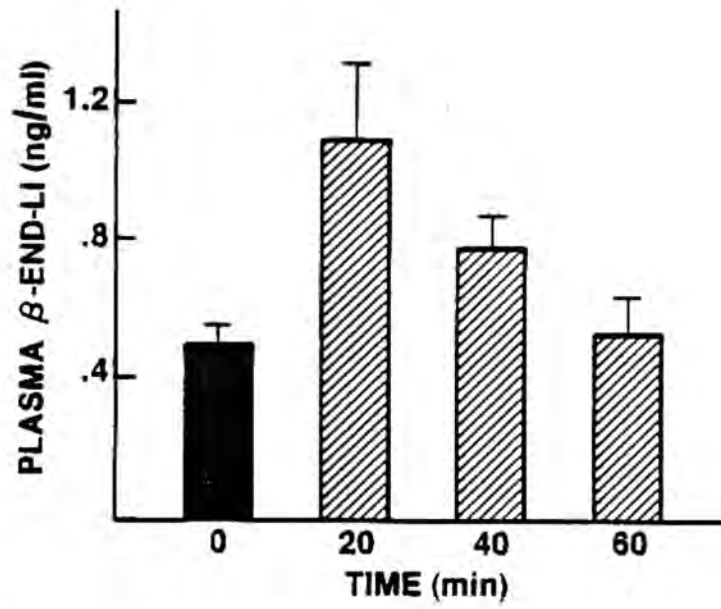
Figure 11

1.11 ± 0.22 ng/ml by 20 min; by 60 min post-injection, circulating levels of β-END-LI had returned to control values (Fig 11). The administration of 25, 75 and 225 mg/kg pargylene elevated basal levels of plasma β-END-LI in a dose-related manner (0.34 ± 0.01 vs. 0.47 ± 0.09 vs. 0.79 ± 0.08 vs. 1.48 ± 0.04 ng/ml) by 45 min post-injection (Fig 11).

In summary, the administration of serotonergic drugs which prolong or mimic the action of serotonin at its post-synaptic receptor, or inhibit serotonin degradation, all resulted in increased levels of plasma β-END-LI. Importantly, the rise in plasma levels of β-END-LI released in response to serotonergic stimulation was significantly attenuated by serotonin receptor antagonists. Thus, the results from these studies further substantiate a stimulatory role of serotonin neurons in the control of pituitary β-END release.

INVESTIGATION OF THE NEURAL BASIS FOR STIMULATED PITUITARY β-END-LI RELEASE

To determine whether serotonin neurons are involved in the stimulated release of pituitary β-END, the effects of serotonergic drugs on plasma levels of β-END-LI released in response to various stimuli were studied. Immobilization and etherization provide reproducible stimuli for the release of pituitary β-END-LI. Furthermore, these stimuli appear to produce varying degrees of stress as evidenced by the observed differences in the magnitude of β-END-LI released. Physical immobilization evokes a near maximal increase in circulating β-END-LI, whereas, etherization produces a more moderate response (Mueller, 1981). Since physical immobilization results in the greatest release of β-END-LI, it provides a good model for examining the effects of serotonergic manipulations thought to inhibit pituitary β-END-LI release. Conversely, etherization

provides a submaximal release of pituitary β-END-LI which may be potentiated following the administration of serotonergic drugs known to stimulate pituitary β-END-LI release.

Immobilization stress evoked a 12-fold rise in plasma β-END-LI levels as compared to non-stressed control values (0.28 ± 0.02 vs. 3.28 ± 0.20 ng/ml; Fig 12, bottom). Five and ten days pretreatment with 75 µg (free base, icv) 5,7-dihydroxytryptamine (5,7-DHT), a serotonin neurotoxin, decreased hypothalamic sertonin content (Fig 12, top) and significantly attenuated the rise in plasma levels of β-END-LI released in response to immobilization stress (Fig 12, bottom). Stress-induced β-END-LI levels in animals treated with 5,7-DHT were 62% and 55% of control stress values 5 and 10 days post-injection, respectively. However, basal levels of circulating β-END-LI were not significantly lowered by this dose of 5,7-DHT after 5 or 10 days treatment. In another experiment using a higher dose of 5,7-DHT (200 µg, free base, icv, for 10 days), significant decreases in levels of both hypothalamic serotonin (1100 ± 41 vs. 434 ± 38 ng/g) and plasma β-END-LI (0.65 ± 0.14 vs. 0.36 ± 0.08 ng/ml) were observed (Fig 13). It should be noted that in this latter experiment, animals treated with 5,7-DHT experienced significant weight loss compared to control animals (average weight of treated animals, 236 ± 15 g vs. a control weight of 298 ± 9 g) and this effect may have contributed to the observed decrease in basal levels of β-END-LI.

Ether stress increased basal levels of plasma β-END-LI from a control value of 0.38 ± 0.02 to 1.08 ± 0.18 ng/ml (Fig 14). Animals pretreated with fluoxetine (10 mg/kg, ip, 15 min) and then exposed to ether (90 sec) had significantly higher levels of plasma β-END-LI than rats exposed to ether alone (1.08 ± 0.18 vs. 1.69 ± 0.09 ng/ml) but not rats exposed

Figure 12. Effects of 5,7-dihydroxytryptamine (5,7-DHT), a sero-
tonin neurotoxin, on hypothalamic serotonin (5-HT) content and plasma
β-END-LI levels in normal and stressed rats. Six groups of rats (n=7-8)
pretreated with desipramine (30 mg/kg, ip, 30 min) to protect catechol-
amine neurons from the neurotoxin, were injected intraventricularly with
either 75 μg 5,7-DHT (free base) or vehicle on day 0 or 5. Animals
injected with vehicle on day 5 served as controls. On day 10, half of
the animals in each group were stressed by restraint for 30 min before
decapitation. Each bar and vertical line represent the mean ± standard
error. Compared to control values, 5,7-DHT treatment significantly
reduced (p<0.05) hypothalamic serotonin content by 75% and 77% 5 and 10
days post-injection, respectively (top). (Hypothalamic sertonin content
was not significantly different between stressed and non-stressed animals
in each of the three groups.) In these same animals, 5,7-DHT treatment
significantly attenuated (p<0.05) the stress-induced increase in plasma
levels of β-END-LI as compared to vehicle-injected controls (bottom).
Basal levels of β-END-LI were not significantly lowered by 5 or 10
days treatment with 5,7-DHT.
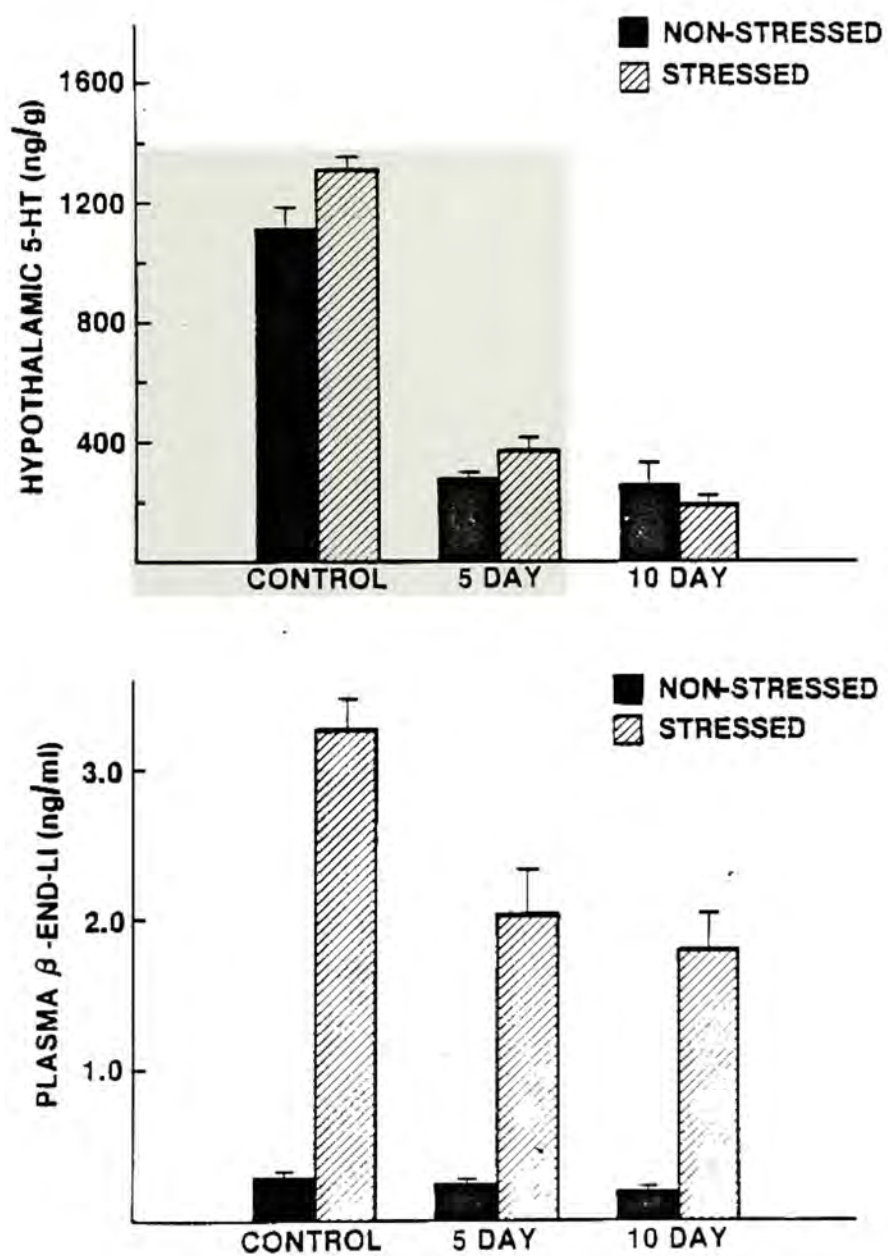
Figure 12

Figure 13. Effects of 200 µg 5,7-dihydroxytryptamine (5,7-DHT), a sero-tonin neurotoxin, on hypothalamic serotonin (5-HT) content and plasma β-END-LI levels. Four groups of rats (n=6-7) pretreated with desipramine (30 mg/kg, ip, for 30 min) to protect catecholamine neurons from the neurotoxin, were injected intraventricularly with either vehicle or 200 µg (free base) 5,7-DHT. Ten days later hypothalami and blood were collected. Each bar and vertical line represent the mean ± standard error. Compared to control values, 5,7-DHT significantly reduced (p<0.05) hypothalamic serotonin content by 60% and plasma β-END-LI levels by 45%.
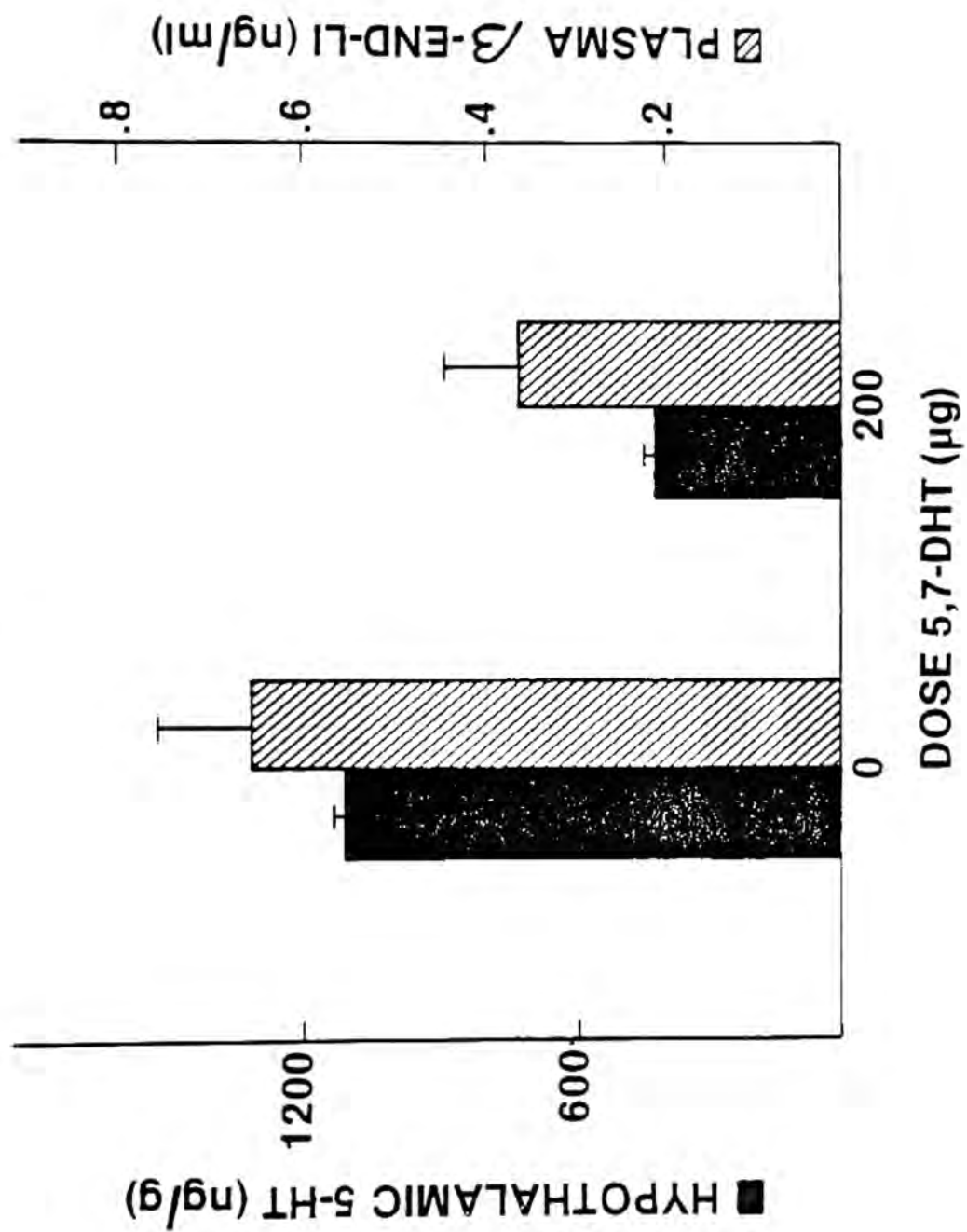
Figure 13

Figure 14. Effects of fluoxetine, a serotonin reuptake blocker,
on basal and stimulated levels of plasma β-END-LI. Two groups of
rats (n=16) were injected ip with either 10 mg/kg fluoxetine or
vehicle, and then half of the animals in each group were immedia-
tely stressed by 90 sec exposure to ether. Blood was collected 15
min after fluoxetine injection. Each bar and vertical line repre-
sent the mean $\pm$ standard error. Compared to vehicle-injected con-
trol values, ether stress or fluoxetine treatment alone significant-
ly increased ($p < 0.05$) plasma levels of β-END-LI. Combined ether-
fluoxetine treatment significantly elevated ($p < 0.05$) plasma levels
of β-END-LI compared to ether stress alone but not fluoxetine
treatment alone.
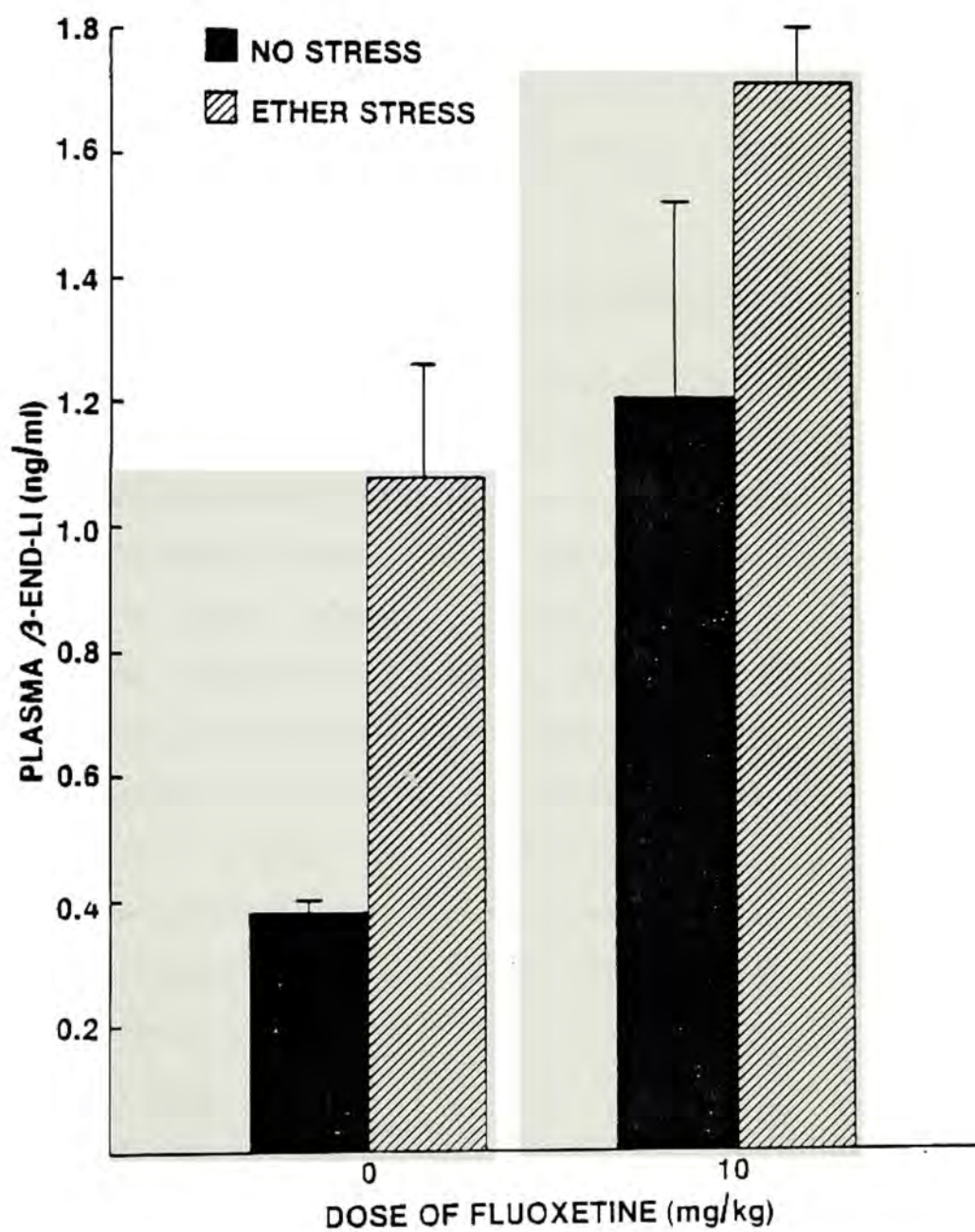
Figure 14

to fluoxetine alone (1.21 ± 0.32 vs. 1.69 ± 0.09 mg/ml; Fig 14).

In summary, pharmacologic destruction of serotonin neurons atten-
uated the plasma β-END-LI response to immobilization stress. Conversely,
indirect stimulation of serotonin receptors with fluoxetine, a drug
which blocks serotonin re-uptake, elevated plasma β-END-LI levels in
etherized rats above those of rats exposed to ether alone. Together
these results suggest that a serotonergic mechanism is involved in the
stress-induced release of pituitary β-END.

DETERMINATION OF THE PITUITARY SOURCE (AL vs. IL) OF BLOOD-BORNE β-END-LI

To determine the pituitary source [anterior lobe (AL) vs. inter-
mediate lobe (IL)] of blood-borne β-END-LI, the gel chromatographic
profiles of immunoreactive β-END released by AL and IL cells in vitro
were compared to those of β-END-LI present in the plasma of rats treated
with serotonergic drugs. Gel filtration profiles of β-END-LI released
from cultured AL and NIL cells show that AL cells secrete two major
forms of β-END-LI corresponding to β-lipotropin (β-LPH) and β-END stan-
dards and contributing 33% and 59%, respectively, to total β-END-LI
secreted (Fig 15). In addition, a small peak eluting at the void
volume was observed and may represent the release of the larger mole-
cular weight precursor. NIL cells, however, released predominantly
one form of β-END-LI resembling β-END standard in molecular size.
These findings, which demonstrate that β-LPH secretion in vitro clearly
distinguishes AL from NIL secretion, suggest that changes in plasma
levels of β-LPH-sized immunoreactivity reflect AL secretion of β-END-LI
in vivo.

Comparison of gel filtration elution profiles from control and

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Figure 15. Comparison of gel chromatographic profiles of β-END-LI released from anterior lobe (AL) or neurointermediate lobe (NIL; pars intermedia plus pars nervosa) cells in culture. Pooled media from AL or NIL cell cultures were chromatographed (Sephadex G-50, fine); arrows indicate the elution volume, purified rat β-lipotropin (β-LPH) and camel β-endorphin$_{1-31}$ (β-END) standards. Void volume is $K_D=0$ and the limit of assay detection is 0.1 ng/fraction.
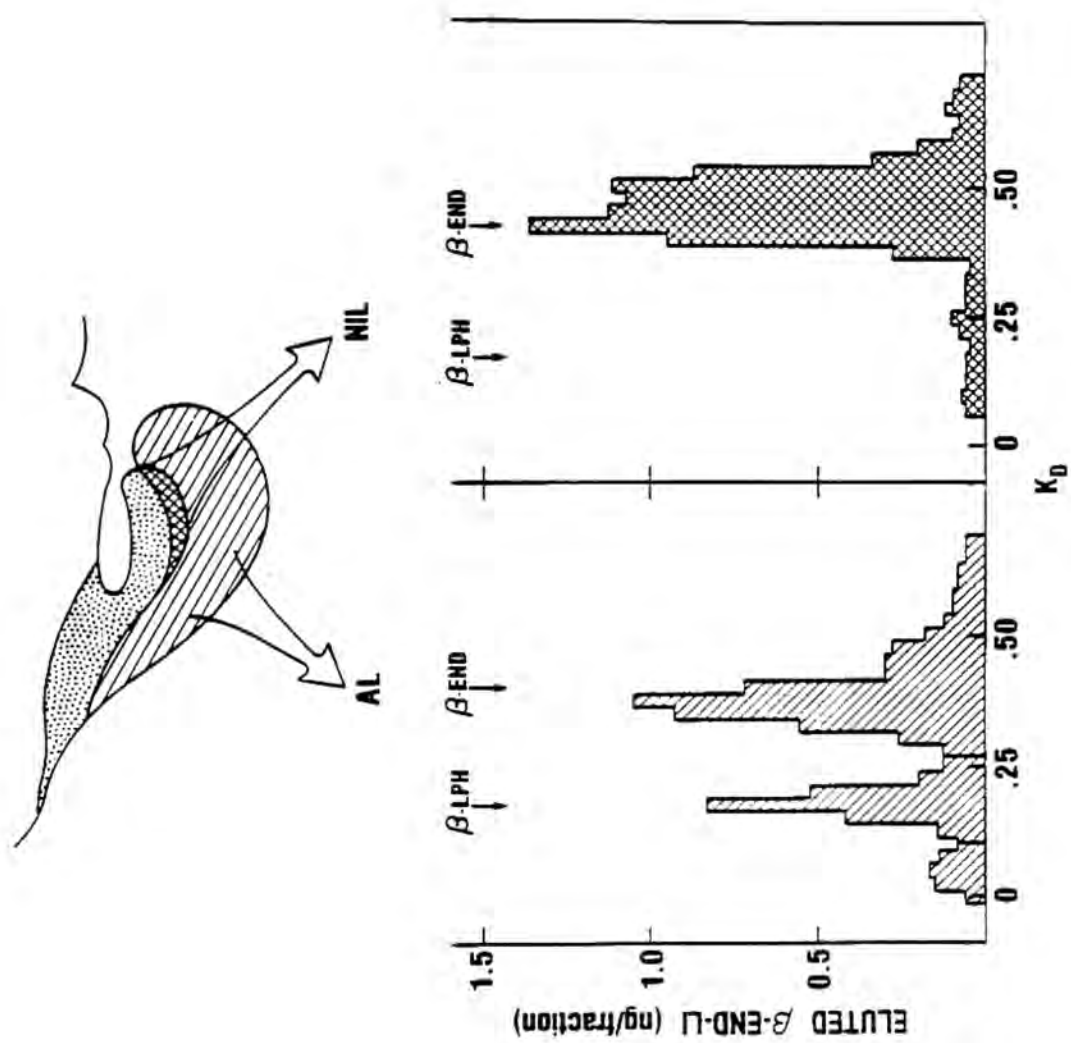
Figure 15

drugtreated animals revealed that TRYP, 5-HTP and fluoxetine, drugs which enhance serotonergic neurotransmission, primarily increased the form of plasma β-END-LI corresponding to β-LPH in molecular size (Fig. 16). In control animals, 20% of the circulating β-END-LI coeluted with β-LPH standard while the remaining 80% coeluted with β-END standard. Following TRYP administration, virtually all (over 90%) of the rise in total β-END-LI was due to the form resembling β-LPH. Similar observations were made with 5-HTP and fluoxetine. Quipazine (5.0 mg/kg), a serotonin receptor agonist, elevated plasma levels of both forms of β-END-LI; however, as seen with TRYP, 5-HTP and fluoxetine treatments, the peak coeluting with β-LPH was primarily affected, being increased 9.5-fold while that resembling β-END was increased less than 1-fold (Fig 17). Similar increases in both β-LPH and β-END peaks (600% vs. 38%, respectively) were observed following quipazine treatment at the lower dose (2.5 mg/kg).

Blockade of serotonin receptors with cinanserin prevented the release of both β-LPH- and β-END-sized immunoreactivity in quipazine-treated animals without significantly decreasing total levels of β-END-LI or altering the ratio of the molecular forms under basal conditions (Fig 18).

Depletion of hypothalamic serotonin content by 77% with 5,7-DHT reduced by 45% the stress-induced release of β-END-LI (Fig 16). Gel filtration chromatography revealed that 5,7-DHT treatment lowered both β-LPH- and β-ENDsized immunoreactivity about equally in stressed animals without significantly affecting the low plasma levels of β-END-LI observed in non-stressed animals (Fig 19).

Immobilization stress dramatically elevated circulating levels

Figure 16. Effects of tryptophan (TRYP), 5-hydroxytryptophan (5-HTP) and fluoxetine on levels and gel chromatographic profiles of plasma β-END-LI. Saline (control), TYRP (200 mg/kg, ip, 30 min), 5-HTP (30 mg/kg, ip, 30 min) or fluoxetine (10 mg/kg, ip, 15 min) were administered to groups of rats (n=8). TRYP and 5-HTP treatment significantly elevated brain levels of serotonin (Table 2, Fig. 5). Levels of total plasma β-END-LI are shown in the top panels where bars and vertical lines represent group means ± standard error. Each chromatogram (bottom panel) depicts the plasma elution profile (Sephadex G-50, fine) of the above treatment group (plasma) pool (8 ml). The elution position for purified rat β-LPH and camel βEND$_{1-31}$ standard are indicated by arrows; void volume is K_D=0 and the limit of assay detection is 0.1 ng/fraction. A comparison of gel filtration elution profiles from control and drug-treated animals shows that TRYP, 5-HTP and fluoxetine primarily increase the molecular form of β-END-LI in plasma corresponding to β-LPH in molecular size.
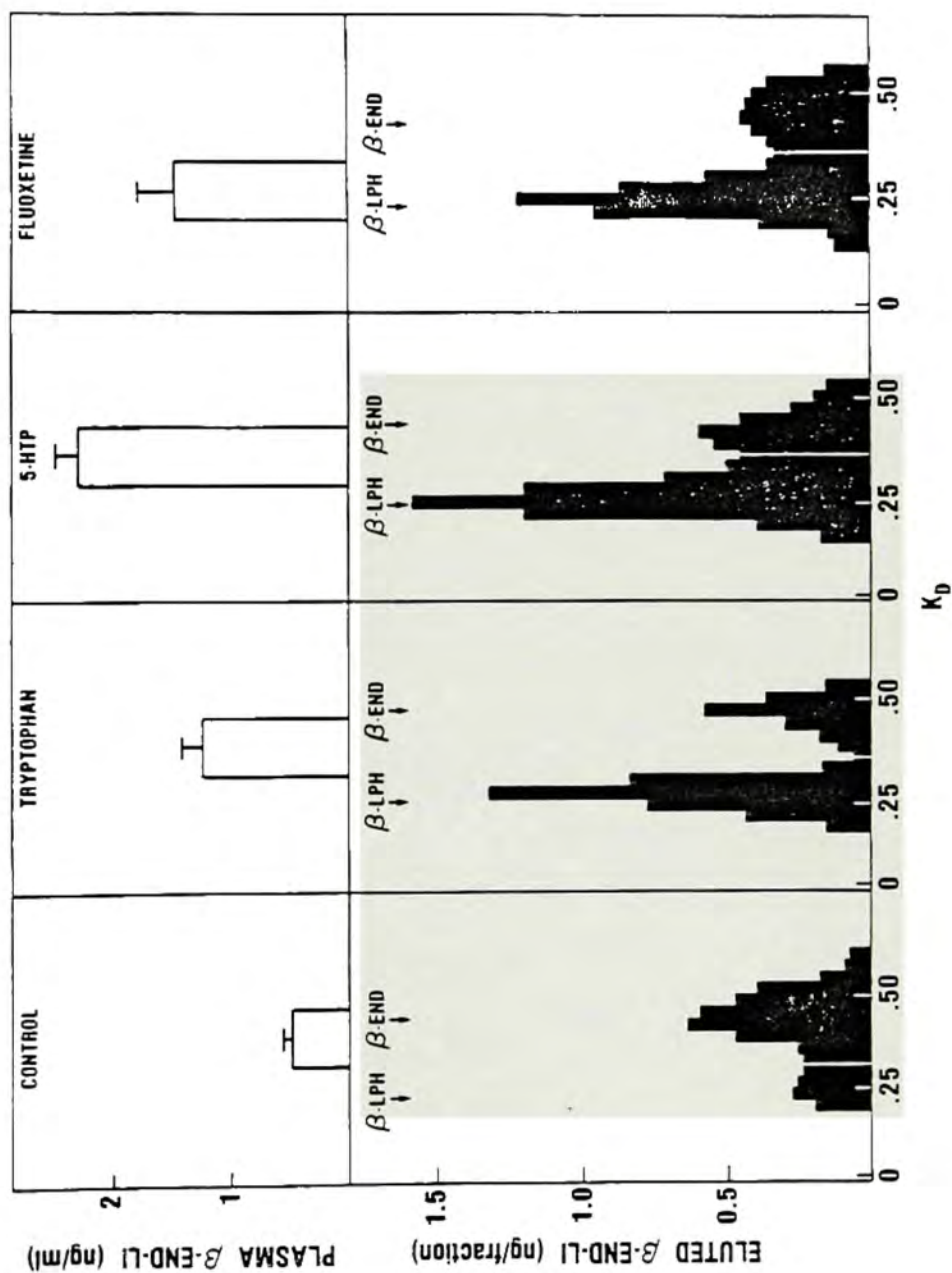
Figure 16

Figure 17. Dose response effects of quipazine on levels and gel chromato-
graphic profiles of plasma β-END-LI. Saline, 2.5 mg/kg or 5.0 mg/kg quip-
azine was administered ip to animals (n=8) 30 min prior to blood collection.
Each chromatogram depicts the plasma elution profile (Sephadex G-50, fine)
of the treatment group above. For details, see text of Fig. 16. A com-
parison of gel filtration elution profiles from control and quipazine-treated
animals show that quipazine at both doses tested elevated both forms of
plasma β-END-LI. However, the immunoreactive peak co-eluting with β-LPH
standard was primarily affected.
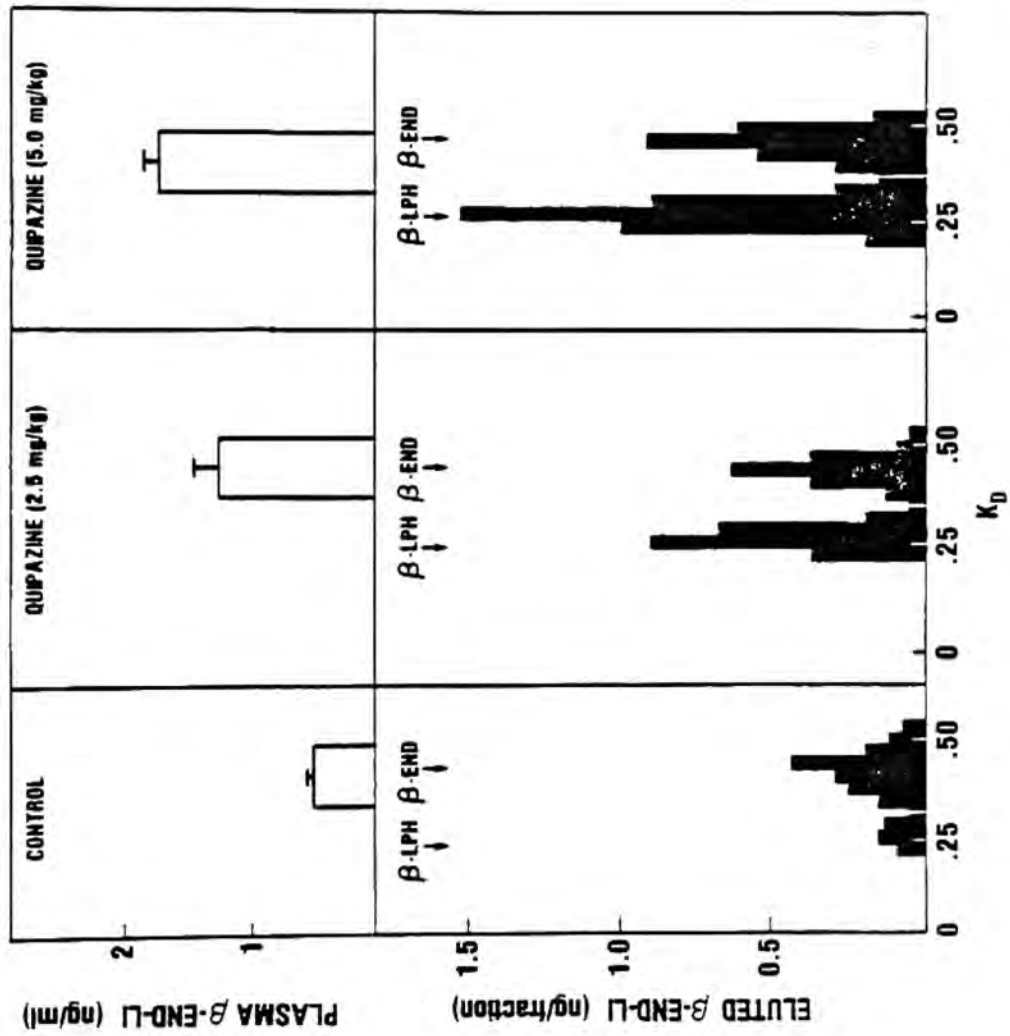
Figure 17

Figure 18. Effects of serotonin receptor blockade with cinanserin on basal and quipazine-stimulated levels and gel chromatographic elution profiles of plasma β-END-LI. Animals were pretreated with either saline or cinanserin (5 mg/kg, ip, 60 min) prior to quipazine treatment (5 mg/kg, ip, 30 min). Each chromatogram depicts the plasma elution profile (Sephadex G-50, fine) of the above treatment group (n=8). For details, see text of Fig. 16. A comparison of gel filtration elution profiles from control and drug-treated animals shows that cinanserin prevented the release of both β-LPH- and β-END-sized immunoreactivity in quipazine-treated animals without significantly (p<0.05) decreasing total levels of β-END-LI or altering the ratio of the molecular forms under basal conditions.
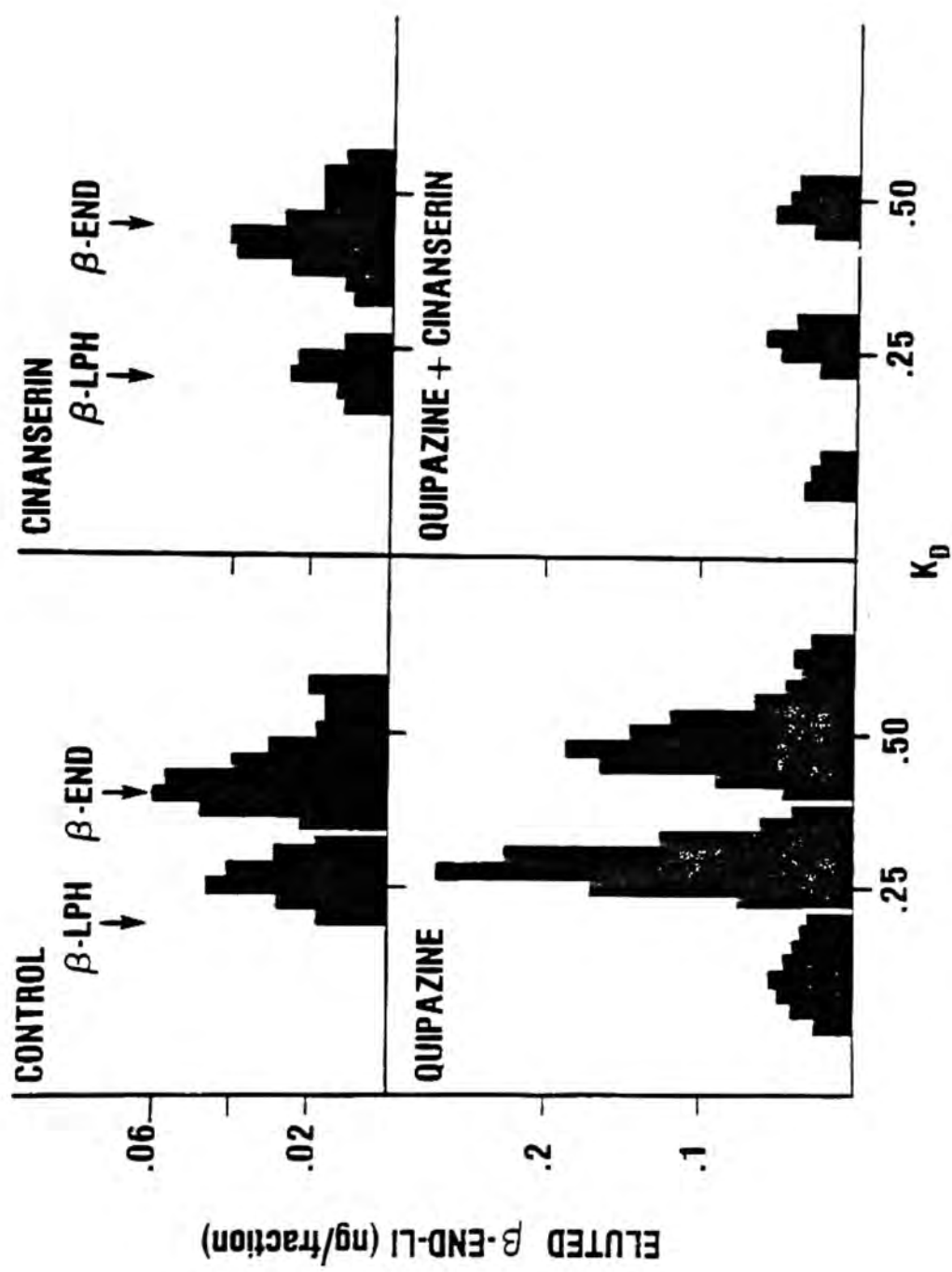
Figure 18

Figure 19. Effects of 5,7-dihydroxytryptamine (5,7-DHT) on levels and gel chromatographic profiles of plasma β-END-LI in normal and stressed rats. Two groups of rats pretreated with desipramine (30 mg/kg, ip, 30 min), to protect catecholamine neurons from the neurotoxin, were injected with either 5,7-DHT (75 µg, free base, icv) or vehicle. Ten days later half of the animals in each group were stressed by immobilization for 30 min before decapitation. Ten days treatment with 5,7-DHT reduced hypothalamic serotonin content by 77% (see Fig. 12). Each chromatogram depicts the plasma elution profile (Sephadex G-50, fine) of the above treatment group (n=7-8). For details, see text of Fig. 16. A comparison of gel filtration elution profiles of plasma from control and drug or stress-treated animals shows that 5,7-DHT treatment lowered both β-LPH and β-END-sized immunoreactivity about equally in stressed animals without significantly affecting the low plasma levels of β-END-LI observed in non-stressed animals.
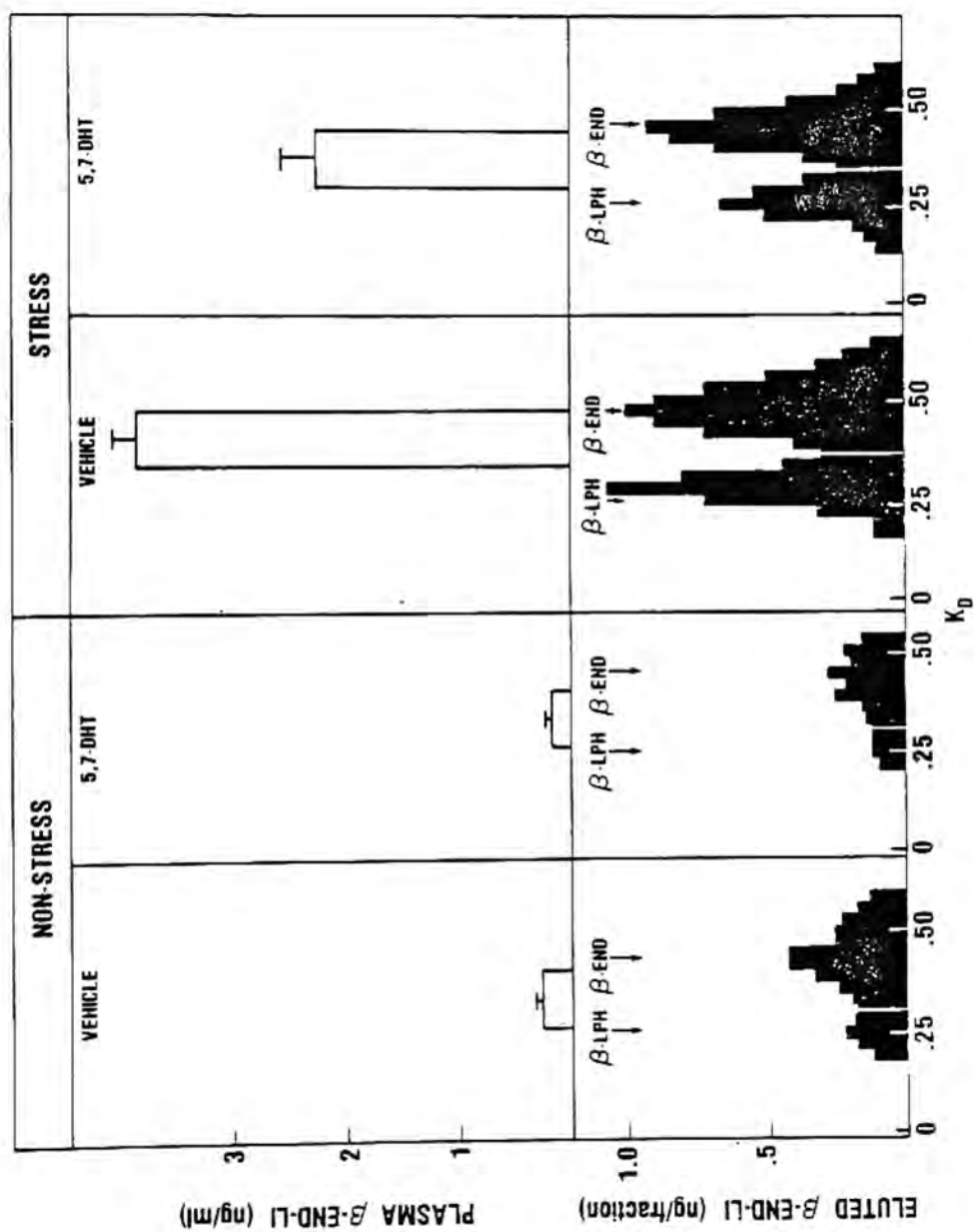
Figure 19

of β-LPHand β-END-sized immunoreactivity, however, there was a greater relative increase in immunoreactivity resembling β-LPH than β-END (4.5-fold vs. 2.5-fold; Fig 19). Similar to immobilization stress, ether stress elevated both molecular forms of β-END-LI with a greater relative rise in β-LPH-sized than β-END-sized immunoreactivity (4.9-fold vs. 1.2-fold; Fig 20). These findings are consistent with the hypothesis that stress stimulates anterior lobe corticotroph secretions (Guillemin et al., 1977; Przewlocki et al., 1982). In addition, it was observed that under conditions of stress a significant portion of total plasma β-END-LI resembled β-END in molecular size suggesting at at least partial release of β-END-LI from the intermediate lobe. In support of this view, it has been reported that α-MSH, a secretory product released along with β-END peptides from intermediate lobe melanotrophs (Mains and Eipper, 1976), is elevated in plasma of stressed rats (Mialhe and Briaud, 1977; Tilders et al., 1982).

To further substantiate serotonergic influence on AL β-END-LI release the effects of dexamethasone, a synthetic glucocorticoid which selectively inhibits AL corticotroph secretion in vitro (Vale et al., 1978, 1979), on plasma levels and gel chromatographic profiles of β-END-LI were studied. Dexamethasone treatment (50 µg/kg, ip, 4 hr) prefer-entially lowered circulating levels of β-LPH-sized immunoreactivity to below the limit of assay detection (Fig 21). Furthermore, dexamethasone pretreatment suppressed by 50% (1.65 ± 0.22 vs. 0.78 ± 0.11 ng/ml) the release of β-END-LI in response to fluoxetine treatment. And virtually all of this reduction was due to lowered levels of immunoreactivity re-sembling β-LPH in molecular size further suggesting that the majority of pituitary β-END-LI released in response to fluoxetine treatment ori-

Figure 20. Effects of ether stress on gel chromatographic profiles of plasma β-END-LI in rats. Animals were injected ip with saline and then half of the animals were immediately exposed to 90 sec of ether in a saturated chamber. Blood was collected 15 min after saline injection. Pooled plasma (8 ml) from each treatment group (n=8) was chromatographed (Sephadex G-50, fine). Arrows indicate the elution positions for purified rat β-LPH and camel β-END$_{1-31}$ standards; void volume is $K_D=0$ and the limit of assay detection is 0.1 ng/fraction. A comparison of gel filtration elution profiles of plasma from control and ether-stressed animals shows that etherization elevated both molecular forms of β-END-LI with a greater relative rise in β-LPH-sized than β-END-sized immunoreactivity (4.9-fold vs. 1.2-fold).
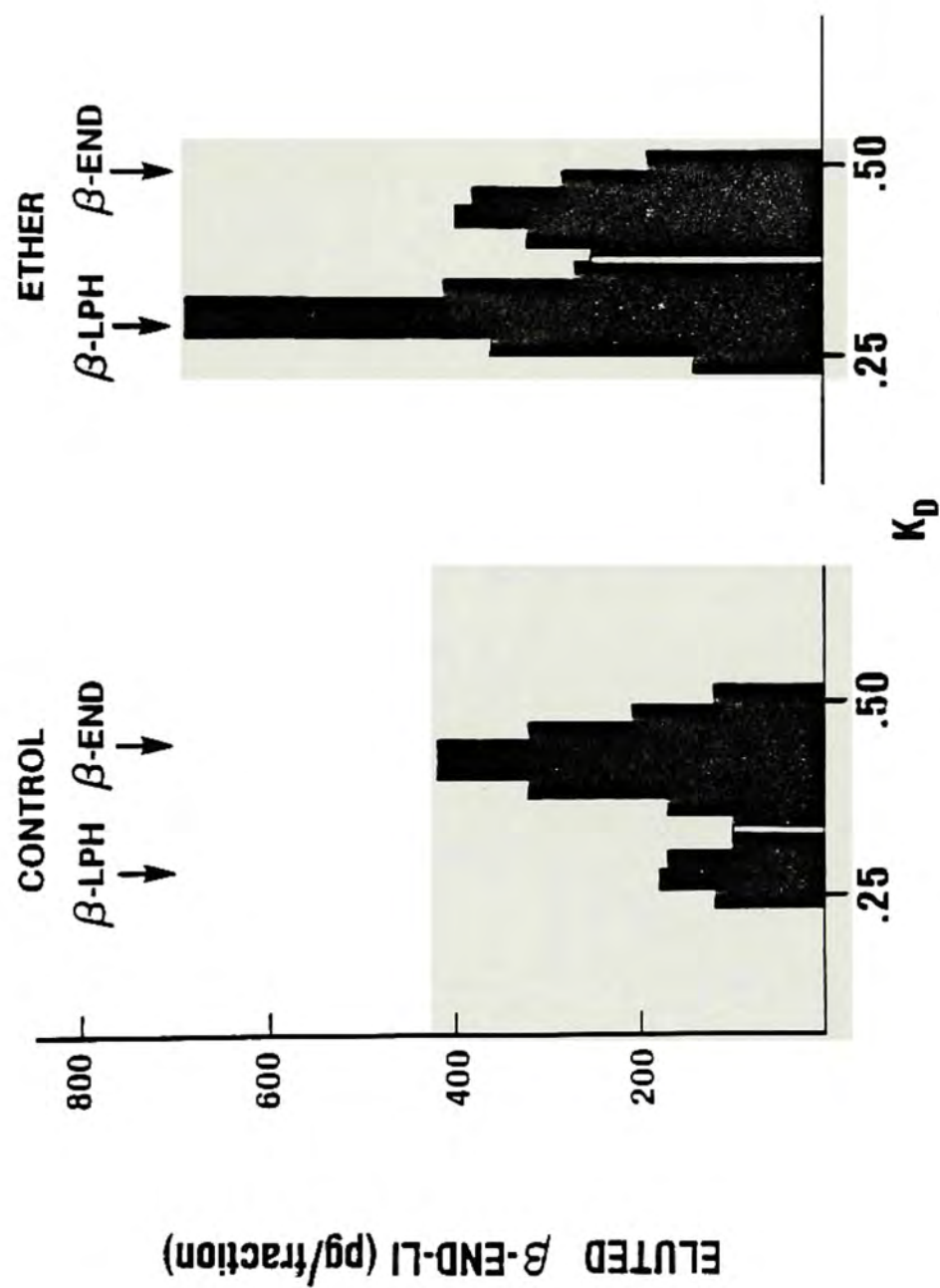
Figure 20

Figure 21. Effects of dexamethasone, a synthetic glucocorticoid, on

levels and gel chromatographic profiles of plasma β-END-LI in control

and fluoxetine-treated animals. Animals were pretreated with either

saline or dexamethasone (50 μ ip, 4h) prior to fluoxetine treatment

(10 mg/kg, ip, 15 min). Blood was collected 15 min after fluoxetine

treatment. Each chromatogram depicts the plasma elution profile

(Sephadex G-50, fine) from the above treatment group (n=8). For details,

see text of Fig. 16. Dexamethasone treatment suppressed by 50% the

fluoxetine-induced rise in plasma β-END-LI levels without significantly

lowering basal levels of plasma β-END-LI at this dose. A comparison of

gel filtration elution profiles of plasma from control and drug-treated

animals shows that dexamethasone treatment preferentially lowered circu-

lating levels of β-LPH-sized immunoreactivity in control and fluoxetine-

treated animals.
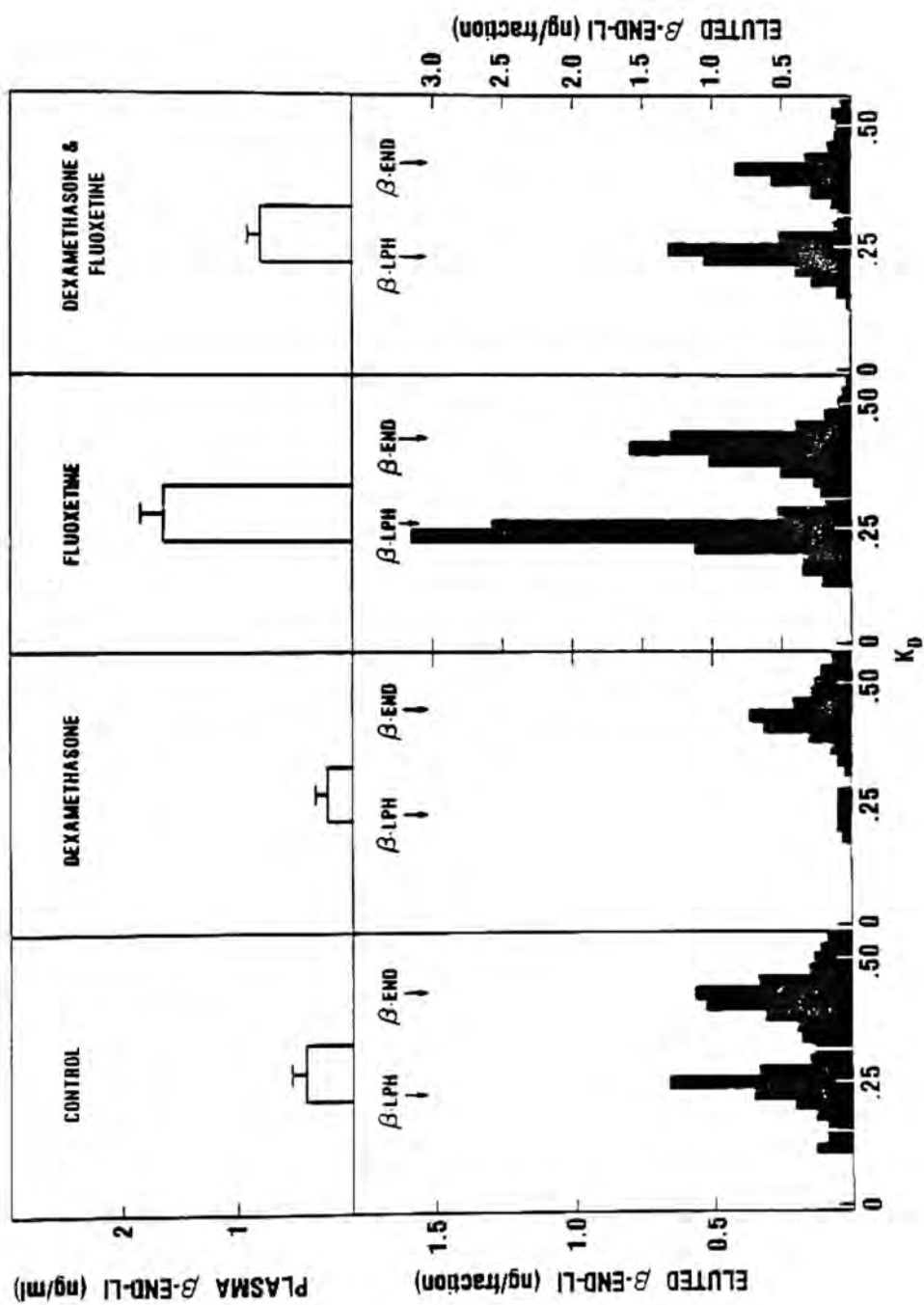
Figure 21

ginates from AL corticotrophs.

In summary, gel filtration chromatography has revealed that serotonergic precursors and serotonergic drugs which stimulate pituitary β-END-LI release predominantly influence the release of β-END-LI resembling β-LPH in molecular size. Furthermore, pretreatment with dexamethasone, a synthetic glucocorticoid, blocked the serotonergic-induced release of β-END-LI. These findings suggest that serotonin neurons preferentially stimulate the release of β-END-LI from the AL in vivo since only the AL secretes appreciable amounts of β-LPH-sized immunoreactivity and only the AL is inhibited by glucocorticoids.

In addition, immobilization and ether stress evoked the release of both molecular forms of β-END-LI with a greater relative increase in β-LPH-sized than β-END-sized immunoreactivity. These findings suggest that stress induces the release of β-END-LI from both the AL and IL in vivo.

THE EFFECTS OF SEROTONIN AND CORTICOTROPH RELEASING FACTOR (CRF) ON THE IN VITRO RELEASE OF β-END-LI FROM ANTERIOR AND INTERMEDIATE LOBE CELLS

To determine whether serotonergic influence over pituitary β-END-LI secretion is exerted directly by serotonin or mediated through CRF, a β-END releasing factor known to be influenced by serotonin, the effects of serotonin and CRF on the release of β-END-LI from cultured AL and IL cells were investigated.

AL release: The effects of serotonin HCl and CRF on the spontaneous release of β-END-LI from primary cultures of AL and NIL cells are shown in Fig 22 (top). Incubation for 90 min with serotonin at concentrations of 10^{-9}M, 10^{-8}M and 10^{-7}M did not affect the basal release

Figure 22. The effects of corticotropin-releasing factor (CRF) and
serotonin HCl on the spontaneous release of β-END-LI from primary
cultures of anterior (AL) and neurointermediate lobe (NIL; pars inter-
media plus pars nervosa) cells. Cultured cells were established by
the method of Vale et al. (1978) and incubated with either CRF or
serotonin at the indicated concentrations for 90 min. The horizontal
hatched areas represent control values of release (AL: 1.12 ± 0.08
ng/plate; NIL: 1.79 ± 0.48 ng/plate). Each bar and vertical line
represent the group mean $\pm$ stanard error (n=6-8). CRF, at all doses
tested, significantly increased (p<0.05) the release of β-END-LI
from AL but not NIL cells. Serotonin, only at the highest dose
(10^{-6}M), increased (p<0.05) the spontaneous release of β-END-LI from
AL cells. Serotonin did not alter the release of β-END-LI from NIL
cells at both doses tested.
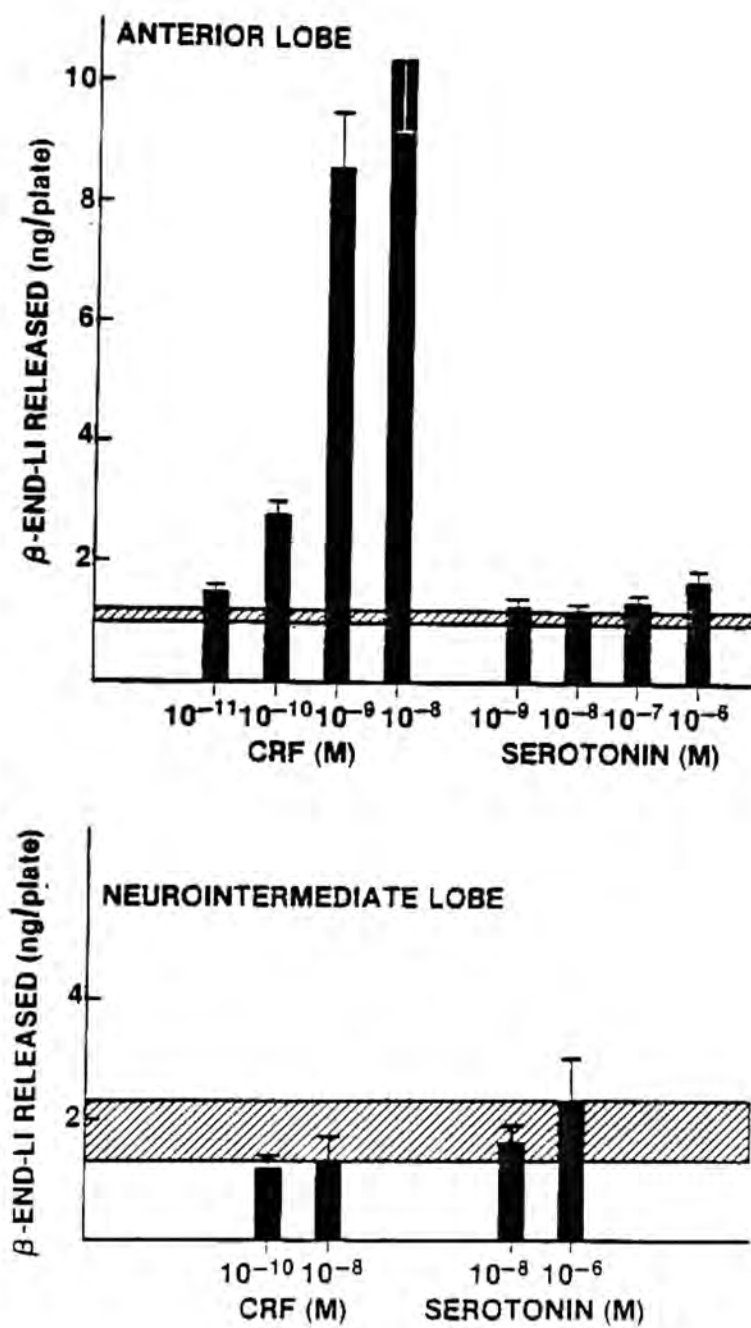
Figure 22

of β-END-LI from anterior lobe cells. A higher concentration of serotonin (10^{-6}M) increased β-END-LI secretion by 53% (1.12 ± 0.08 vs. 1.74 ± 0.12 ng/plate). The effects of this higher dose of serotonin on β-END-LI release may be artifactual since the drug preparation contains an equimolar concentration of HCl which may exert an effect of its own on AL corticotrophs at this high concentration (10^{-6}M). To control for this, the effects of 10^6M HCl on the release of β-END-LI from cultured cultured AL cells should have been examined.

CRF, a known β-END releasing factor, stimulated the release of β-END-LI from AL cells in a dose-related manner (Fig 22, top). CRF at a concentration of 10^{-9}M and 10^{-8}M increased β-END-LI release 7.7-fold and 9.2-fold, respectively, over basal release (1.12 ∓ 0.08 ng/plate). Lower concentrations of CRF (10^{-11}M and 10^{-10}M) tended to increase β-END-LI release, however, these increases were not statistically significant from basal release levels.

NIL release: As shown in Fig 22 (bottom), serotonin at concentrations of 10^{-8}M and 10^{-6}M did not alter the in vitro release of β-END-LI from the NIL. Likewise, CRF (10^{-10}M or 10^{-8}M) did not significantly alter the release of β-END-LI from cultured NIL cells following 90 minutes of incubation).

In summary, CRF evoked a dose-related increase in the release of β-END-LI from cultured AL but not NIL cells. Serotonin did not significantly alter the in vitro release of β-END-LI from either AL or NIL cells, except at the highest dose (10^{-6}M), serotonin increased the spontaneous release of β-END-LI from AL but not NIL cells. These findings suggest that serotonergic regulation of pituitary β-END-LI release is most likely mediated through a β-END releasing factor rather than a direct effect of the neurotransmitter on the pituitary.

THE EFFECTS OF ELECTRICAL STIMULATION OF BRAINSTEM SEROTONERGIC NUCLEI

ON PITUITARY β-END-LI RELEASE

Serotonergic cell bodies of the Dorsal Raphe Nucleus (DRN) and Median Raphe Nucleus (MRN) project to hypothalamic areas known to be involved in the neuroendocrine regulation of pituitary hormone release. If serotonin neurons regulate pituitary β-END secretion then electrical activation of the raphe serotonergic nuclei in vivo should result in an increase in plasma levels of β-END-LI. To further study the role of serotonergic neurons in the regulation of pituitary β-END-LI release, the effects of DRN and MRN electrical stimulation on plasma levels of β-END-LI were studied. Data presented here was obtained from animals in which electrode placement in the DRN and MRN was verified by electrode marking and subsequent histology (not shown).

Initial studies were carried out to determine the optimal stimulus parameters and time course for blood collection. In addition, it was determined that withdrawal of 0.5 ml samples of blood with immediate re-infusion of 0.5 ml of 0.9% NaCl had virtually no effect on circulating levels of β-END-LI (not shown). Plasma levels of β-END-LI did not deviate more than 3% from control values during the five samplings obtained over a 25 min time course. Moreover, sampling at five minute intervals appeared to have no effect on plasma levels of β-END-LI. In all subsequent experiments, 0.5 ml blood samples were withdrawn with 0.5 ml saline reinfused immediately following blood withdrawal to assure that the observed effects are due to the treatment alone and not due to a loss of blood volume.

Stimulus Current Dose-Response: Table 3

Compared to pre-stimulation control values (0 mA stimulus strength),

Table 3. Electrical stimulation of the Dorsal Raphe Nuclei:
Effects of increasing stimulus current intensity on plasma
levels of β-END-LI

Table 3

Current (mA)	Plasma β-END-LI (ng/ml)
Control	1.53 ± 0.48
0.05	1.54 ± 0.46
0.20	1.65 ± 0.51
0.50	1.96 ± 0.54

Constant current, biphasic pulse (0.2 ms duration), 10 pulses per sec, 2 min stimulation, n=2

two minute DRN stimulation with increasing stimulus strength (0.05 mA – 0.5 mA) had no significant effect on plasma levels of β-END-LI at a stimulus pulse duration of 0.2 ms. Applying a stimulus intensity greater than 0.5 mA or a stimulus pulse duration of greater than 1.0 ms caused visible whisker and eye lid twitching suggesting activation of CNS motor neurons.

Stimulus Time Course: Table 4

Compared to pre-stimulation control values, 2, 5 and 10 minutes of electrical stimulation of the DRN (0.3 mA; 1 ms) tended to increase circulating levels of β-END-LI. By 15 minutes after stimulation was terminated, plasma levels of β-END-LI remained elevated as compared to pre-stimulation values and tended to be higher than those observed at 10 minutes of stimulation. However, due to the large standard error associated with each mean, these changes in plasma levels of β-END-LI were not statistically different from control values or from each other (One-way analysis of variance, $p > 0.05$).

Electrical Stimulation of the DRN vs. MRN: Fig. 23

Compared to non-stimulated control values, as well as pre-stimulation plasma β-END-LI values, 15 minutes of electrical stimulation (0.3 mA; 0.3 ms) of the DRN or MRN had no significant effect on plasma levels of β-END-LI. In DRN-stimulated animals, plasma levels of β-END-LI changed very little from pre-stimulation levels of 3.25 ± 0.24 ng/ml to 2.90 ± 0.29 ng/ml β-END-LI at 15 minutes of stimulation. Likewise, in MRN-stimulated animals, plasma levels of β-END-LI at 15 minutes of stimulation (2.99 ± 0.39 ng/ml) were unchanged from pre-stimulation levels (3.12 ± 0.36 ng/ml). In addition, in both DRN- and MRN-stimulated animals, plasma levels of β-END-LI were within the range

Table 4. Electrical stimulation of the Dorsal Raphe Nuclei:

Effects of electrical stimulation on levels of plasma β-END-LI

over time

Table 4

Time (min)	Plasma β-END-LI (ng/ml)
0	1.76 ± 0.30
2	2.31 ± 0.39
5	2.48 ± 0.44
10	2.61 ± 0.42
25	3.05 ± 0.49

Constant current (0.3 mA), biphasic pulse (1 ms. duration), 10 pulses per second, n=1

Figure 23. The effects of electrical stimulation of the Dorsal (DRN) and Median Raphe Nucleus (MRN) on plasma levels of β-END-LI in rats. Rats were anesthetized with a urethane (1.3 g/kg, ip)-α-chloralose (95 mg/kg, ip) solution. A concentric bipolar electrode was placed stereotaxically in the MRN or DRN and a biphasic (0.3 msec duration) constant current (0.3 mA) stimulus (10 pulses/sec) was delivered for 15 min. Control animals were treated similarly but no current was passed through the electrode. Blood was withdrawn from a femoral artery cannulation at 5 and 10 min prior and 5, 10 and 15 min during electrical stimulation. Following blood withdrawal an equal volume (0.5 ml) of saline was reinfused through the femoral catheter. Each point and vertical line represent the group mean ± standard error (n=3). Compared to non-stimulated control values and pre-stimulation plasma β-END-LI values, 15 min of electrical stimulation of the DRN or MRN had no significant effect on plasma levels of β-END-LI in anethetized rats.

Figure 23

of β-END-LI levels observed in non-stimulated control animals over the same time period.

In summary, the results from the neurophysiologic studies suggest that raphe serotonin neurons exert little, if any, influence over the secretion of pituitary β-END-LI in anesthetized rats. rats. Electrical stimulation of the DRN did not significantly alter circulating levels of β-END-LI over a 10-fold increase in current strength or during a 15 minute period of stimulation. Likewise, 15 minutes of stimulation of the MRN at a maximal current strength and duration which did not produce visible motor responses (i.e. whisker or eyelid twitching) had no effect on plasma levels of β-END-LI. It is important to note here that pre-stimulation and control plasma levels of β-END-LI were extremely high (3.5 ± 0.2 ng/ml; Fig. 23) as compared to plasma β-END-LI levels obtained by rapid decapitation from unanesthetized, untreated rats (0.30 ± 0.06 ng/ml; Table 2). It may be thus concluded that anesthesia and surgical stress, which evoked a large rise in plasma β-END-LI levels, prevented or masked the subsequent effects of electrical stimulation. A better model for studying the effects of raphe electrical stimulation on pituitary β-END release should include unanesthetized, unrestrained rats with implanted electrodes for electrical stimulation and indwelling catheters for blood collection.

DISCUSSION

Overall, the results presented here indicate that brain sero-
tonin neurons exert a stimulatory influence over the basal secretion of
pituitary β-END-LI and are involved, at least in part, in the stress-
induced release of this hormone in rats. Moreover, serotonin neurons
appear to preferentially stimulate β-END-LI release from the anterior
lobe of the pituitary in vivo. Evidence from the in vitro studies suggest
that serotonergic regulation of anterior lobe β-END-LI release is most
likely mediated through a hypothalamic β-endorphin releasing factor,
probably CRF.

THE EFFECTS OF SEROTONIN PRECURSORS ON PLASMA LEVELS OF β-END-LI

The rate at which serotonin is synthesized in brain is depend-
ent on the availability of its primary substrate, L-tryptophan, and the
enzymes and co-factors responsible for the subsequent conversion of tryp-
tophan to serotonin. Since tryptophan hydroxylase, the rate-limiting
enzyme which hydroxylates tryptophan to 5-hydroxytryptophan (5-HTP), is
unsaturated by its substrate (the k_m for tryptophan exceeds normal tryp-
tophan concentrations in brain), changes in plasma and brain levels of
tryptophan can be expected to influence serotonin synthesis. In support
of this, it has been shown that tryptophan and serotonin increase brain
serotonin synthesis (Grahame-Smith, 1971; Fernstrom and Wurtman, 1971) and
hypothalamic serotonin metabolism (Mueller et al., 1976) suggesting the
enhancement of serotonin neurotransmission.

The results presented here demonstrate that doses of tryptophan
and serotonin which significantly elevated brain serotonin content also

elevated circulating levels of β-END-LI (Table 2; Fig. 5). Assuming
that increased serotonin synthesis and content lead to enhanced serotonin
release, the results suggest that serotonin neurons exert a stimulatory
influence over pituitary β-endorphin release. It was also observed
that 5-HTP elevated brain serotonin content and plasma β-END-LI levels
to a greater extent than tryptophan. An explanation for this observation
is that 5-HTP is a more immediate precursor than tryptophan with a faster
conversion rate to serotonin. The difference may also be partially due to
the less specific action of 5-HTP as compared to tryptophan. Unlike tryp-
tophan which is hydroxylated within only serotonin neurons (Aghajanian and
Asher, 1971), 5-HTP can cause serotonin to accumulate in non-serotonergic
nerve terminals, such as dopaminergic neurons (Butcher et al., 1972),
which contain the decarboxylase enzyme necessary to convert 5-HTP to sero-
tonin. Since dopaminergic neurons can exert an inhibitory influence over
the secretion of β-END-LI in rats (Farah et al., 1982; Giraud et al.,
1980), 5-HTP treatment may stimulate β-ENDLI release through a combinated
action to enhance serotonergic neurotransmission and inhibit dopaminergic
neurotransmission. In addition, it is possible that the high levels of
tryptophan in plasma following tryptophan administration (200 mg/kg) may
competitively reduce the ability of other neutral amino acids (i.e. tyro-
sine, a precursor for catecholamine biosynthesis) to gain access into the
CNS. Blood-brain barrier transport of tryptophan and other neutral amino
acids is known to be influenced by substrate competition (Pardridge, 1979),
thus the net effect of enhanced serotonin neurotransmission may be offset
by the simultaneous disruption of neurotransmission by other biogenic
amine pathways.

One further point with regard to the difference between trypto-

phan and 5-HTP's ability to raise plasma levels of β-END-LI. Both
systemic and local applications of tryptophan and 5-HTP have been shown to
depress raphe serotonergic firing (Gallager and Aghajanian, 1976), indica-
ting that both precursors are able to sufficiently increase serotonin
release to initiate negative feedback. Furthermore, it was noted that
tryptophan maximally depressed raphe firing by 15-20 min after adminis-
tration, whereas the inhibition induced by 5-HTP developed more slowly,
requiring 30-40 minutes for maximal inhibition to occur. Thus, 5-HTP's
ability to further increase plasma βEND-LI than tryptophan may be due,
in part, to the longer delay observed for the inhibition of serotonin
neuron firing to occur following serotonin administration.

The observation that a smaller dose of tryptophan (75 versus 200
mg/kg) elevated plasma levels of β-END-LI without significantly elevating
total brain serotonin content may be best explained on the basis that
total levels of brain serotonin may not necessarily correlate with sero-
tonergic function. A better index of serotonergic activity may be the
measurement of changes in serotonin levels reflecting serotonergic meta-
bolism. Also, the slight increase in total brain serotonin content may
mask a significant increase in regional (hypothalamic) serotonin function
and activity which may result in increased serotonergic stimulation of
pituitary β-END-LI secretion. Further, there is evidence to suggest
that serotonin synthesis and storage occurs in separate neuronal pools,
one being the "storage pool" and the other being the "releaseable pool"
(Shields and Eccleston, 1973). Serotonin within the "releaseable pool"
represents the newly-synthesized amine which is released by the nerve
terminal. In contrast, serotonin in the storage pool has been postulated
to be synthesized by a separate mechanism which is not controlled by neur-

onal activity. Thus, precursor loading with tryptophan may stimulate the synthesis and release of serotonin from a small, but functional, pool without affecting the larger storage pool and total brain levels of serotonin.

Despite the inherent difficulties in using tryptophan and 5-HTP to selectively increase serotonergic function, precursor loading is thought to be the most "physiologic" approach to manipulate serotonin neurons. (Peripheral administration of serotonin has little, if any, effect on CNS function since it is rapidly degraded by blood and tissue enzymes, as well as, enzymes within the the endothelium of the blood-brain barrier). In general, the findings presented here suggest that serotonin neurons exert a stimulatory influence over the secretion of pituitary β-endorphin as judged by the dose- and time-related increases in plasma levels of β-END-LI following serotonin precursor administration.

EFFECTS OF SEROTONERGIC DRUGS ON PLASMA LEVELS OF β-END-LI

The administration of drugs which prolong or mimic the action of serotonin at its post-synaptic receptor all resulted in increased plasma levels of β-END-LI. These results further support the hypothesis that serotonin neurons exert a stimulatory influence over the secretion of pituitary β-endorphin. Of course, an assessment of serotonergic influence on pituitary β-END-LI secretion based on a phamacologic approach is dependent on the specificity and potency of the drugs employed. Unfortunately, no drug is absolutely pure and selective for one receptor or substrate. Thus, the possiblity of serotonergic drug interaction with other neurotransmitter systems must always be considered as a possi-

ble additional influence on pituitary secretion. However, since doses of

serotonin receptor active drugs have been carefully chosen here so that

little, if any, cross-reaction with other neurotransmitter systems occurs,

the effects observed here on pituitary β-END-LI secretion are most likely

due to the drug's predominant action on serotonin neurons.

Quipazine is a serotonin receptor agonist which possesses some

antinociceptive properties believed to be a result of its stimulatory

action on central serotonin receptors (Samanin et al., 1976). Quipazine

was shown to significantly increase plasma β-END-LI levels by 15 minutes

post-injection, and by 90 minutes plasma β-END-LI levels had returned to

control values (Fig. 6).

These results suggest that the time course of pituitary β-END-LI

release is acute (within 5 minutes) and that the rate of decline in

plasma levels (between 45 and 60 minutes) most likely reflects the meta-

bolic clearance rate of this peptide from circulation. Houghten et al.,

(1980) have reported that the half-life of human β-endorphin injected

into the bloodstream of rats is 45 minutes. In these studies, the half-

life of rat β-endorphin appears to be much longer (between 60-90 minutes).

It is also possible that the decrease in plasma β-END-LI levels may

represent negative feedback effects of high plasma and/or pituitary con-

centrations of β-END-LI on the hypothalamic factors controlling its

release. β-endorphin has been shown to be present in high concentration

in the pituitary portal vasculature (Lissitzky et al., 1980) and evidence

for retrograde blood flow in the pituitary stalk toward the hypothalamus

has been presented by Oliver et al., (1977). In addition, β-endorphin

has been shown to penetrate the blood-brain barrier at physiological

concentrations (Houghten et al., 1980) and to influence the activity

of neurons involved in the regulation of anterior pituitary hormone release (Van Loon et al., 1980). Thus it is possible that pituitary β-endorphin may regulate its own release by a short-loop negative feedback mechanism similar to other pituitary hormones. In addition, it is possible that a long-loop negative feedback mechanism involving the secretion of hormones from target tissue may be operating to inhibit the further release of pituitary β-END-LI. A likely candidate is adrenal corticosterone, a glucocorticoid known to be released in response to ACTH, a peptide released along with β-END-LI under most if not all conditions (Guillemin et al., 1977). Glucocorticoids have been shown to inhibit β-END-LI secretion from cultured anterior lobe cells (Vale et al., 1979; 1983) and to reduce circulating levels of β-ENDLI (Hollt et al., 1978; Mueller et al., 1981). Since β-END-LI and ACTH share common regulatory mechanisms it is possible that glucocorticoids participate in the negative feedback control of pituitary β-endorphin.

Doubling the dose of quipazine from 5 to 10 mg/kg had no further effect on plasma β-END-LI levels (Fig. 6). This observation suggests that all serotonin receptors involved in the regulation of pituitary β-END-LI were occupied following the 5 mg/kg dose of quipazine and that no further effect could be observed on plasma β-END-LI levels with the higher dose. It is also possible that at the higher dose, quipazine may affect other neurotransmitter systems which interact in a competitive fashion to attenuate the net effect. In support of this alternative, quipazine has been shown to interact with dopamine neuron function at this higher but not lower dose (Ponzio et al., 1981), and dopamine neurons have been shown to inhibit pituitary β-END-LI release (Farah et al., 1982; Giraud et al., 1980).

The ability of cinanserin, a serotonin receptor antagonist, to block the quipazine-induced rise in plasma β-END-LI (Fig. 7) further suggests that quipazine's effect on pituitary β-END-LI secretion is due to serotonin receptor stimulation. Cinanserin treatment alone at various doses and treatment times was unable to reduce circulating levels of β-END-LI (Fig. 8) and this may indicate that the basal secretion of β-endorphin is under tight regulation which prevents a decrease in the already low circulating levels.

Fluoxetine is an interesting and widely used drug which inhibits the reuptake of serotonin by the pre-synaptic nerve terminal and thereby increases the duration of serotonin activity within the synaptic cleft and the possibity of its binding to post-synaptic receptors. Accordingly, fluoxetine's action serves to increase endogenous serotonergic tone by enhancing existing serotonergic neurotransmission. Fluoxetine treatment elevated basal levels of plasma β-END-LI (Fig. 9) indicating that serotonin neurons are involved in the stimulatory regulation of pituitary β-END-LI release. Since cyproheptadine, a serotonin receptor blocker, attenuated the fluoxetine-induced rise in plasma β-END-LI (Fig. 9), this further suggests that fluoxetine's effects on pituitary β-END-LI secretion are mediated through a serotonergic mechanism. Complete blockade of the fluoxetine-induced rise in plasma levels of β-END-LI was not observed by the doses of cyproheptadine tested. However, the observation that increasing doses of cyproheptadine resulted in a greater attenuation of the fluoxetine-induced rise suggest that perhaps not all serotonin receptors were blocked at the doses used. Like cinanserin, cyproheptadine also had no effect on resting levels of plasma β-END-LI.

Another pharmacologic approach for enhancing serotonergic neuro-transmission is to prolong serotonergic activity within the synaptic cleft by inhibiting its enzymatic degradation. Pargylene is a drug which inhibits monoamine oxidase, the enzyme that is principally respon-sible for the metabolism of serotonin to 5-HIAA. Pargylene has been shown to increase intraneuronal stores of serotonin (Tozer et al., 1966) which apparently results in increased concentrations of serotonin in the synaptic cleft due to the release of larger quantities of seroto-nin per nerve impulse. Pargylene treatment was shown to increase plasma β-END-LI concentrations in a dose-related manner (Fig. 11), suggesting once again that serotonin neurons exert a stimulatory influence over the secretion of pituitary β-END-LI. The time course of pituitary β-END-LI release following pargylene treatment shows an acute 2-fold rise (within 20 min) with a peak at 40 min and a return to control levels by 60 min post-injection (Fig. 11). This time course of pituitary β-END-LI release is similar to the one observed following quipazine treatment. β-END-LI is released within 20 min and is cleared from circulation by 90 min.

One important point about pargylene that should be noted is that it inhibits the degradation of other biogenic amine neurotransmitters such as catecholamines which are known to be metabolized by monoamine oxidase. Hence, the effects observed here are not due solely to an in-crease in serotonergic activity but may also be due to enhanced norad-renergic neurotransmission which has also been associated with increased pituitary β-END-LI release (Pettibone and Mueller, 1982). However, pargylene at the times and doses employed here has been reported to significantly increase brain serotonin content and lower 5-HIAA concen-trations (Reinhard et al., 1980) indicating an important effect of

pargylene on serotonin function. Furthermore, the observed effects on plasma levels of β-END-LI following pargylene treatment are consistent with all previous findings in which serotonergic neurotransmission is presumably increased by different methods.

The administration of serotonin, the endogenous ligand for serotonin receptors, provides another pharmacologic method for the enhancement of serotonergic neurotransmission. Since peripherally administered serotonin is rapidly degraded (liver, lung) before significant amounts enter the CNS, serotonin was administered centrally. Since the effects of serotonin on pituitary secretion were expected to be acute, animals were anesthetized 20 min prior to icv injection and remained anesthetized until samples were collected 15 min later. The general anesthetic pentabarbital was used since its minor effect to increase plasma levels of β-END-LI is known to occur within 10 min of injection and is followed by a return of plasma levels of β-END-LI toward control values by 20 min (Mueller and Maiewski, 1983). Thus, at the time of blood collection, changes in blood levels of β-END-LI are most likely due to the effects of exogenous serotonin. Serotonin administration increased circulating levels of β-END-LI in a dose-related manner, however, only at the highest dose was this increase statistically significant (Fig. 10). The large standard error of the mean within each treatment group masked any significant effects of serotonin at the lower doses. A contributing factor to the large spread in plasma β-END-LI values within each treatment group is most probably the use of anesthesia. Anesthesia introduces another variable into each group by producing a stimulation of its own on pituitary β-END-LI. In addition, pentabarbital obviously effects CNS function in order to produce anesthesia and these effects may

influence serotonergic function. Thus the observed changes in plasma levels of β-END-LI may be due to the combined effects of serotonin stimulation and anesthesia on pituitary β-END-LI secretion.

In summary, the administration of serotonergic drugs which prolong or mimic the action of serotonin at its post-synaptic receptor, or inhibit serotonin degradation all resulted in increased levels of plasma β-END-LI. These results are consistent with the serotonin precursor studies and further substantiate a stimulatory role of serotonin neurons in the control of pituitary β-endorphin release.

THE EFFECTS OF ELECTRICAL STIMULATION OF BRAINSTEM SEROTONERGIC NUCLEI ON PITUITARY β-END-LI RELEASE

It is well established that serotonergic neurons of the raphe nuclei play an important role in pain perception and analgesia (see Messing and Lytle, 1977; Mayer and Price, 1978). Electrophysiologic stimulation of raphe nuclei produces a behavioral analgesia in both laboratory animals and man. In fact, electrical stimulation of the raphe region in humans ameliorates otherwise intractable pain caused by a variety of pathologies. Conversely, destruction of raphe nuclei results in hyperalgesia which can be reversed upon restoring serotonergic function. Interestingly, the serotonergic cell bodies of the raphe nuclei (Dorsal and Median) project to hypothalamic areas known to be involved in the neuroendocrine regulation of pituitary hormone release (van De Kar and Lorens, 1979) and may well evoke the release of pituitary β-endorphin when electrically stimulated. Accordingly, release of pituitary β-endorphin may participate in the analgesia associated with raphe stimulation.

To examine the question of whether raphe serotonin neurons are in-

volved in the regulation of pituitary β-endorphin release, a series of
experiments were conducted in rats in which the Dorsal (DRN) and Median
(MRN) raphe nuclei were electrically stimulated and blood was collected
to determine the effects of stimulation on plasma levels of β-END-LI.
It was observed that electrical stimulation of the DRN did not signifi-
cantly alter circulating levels of β-END-LI over a 10-fold increase in
current strength or during a 25 min period of stimulation (Table 3 and 4).
Likewise, 15 min of MRN stimulation at a maximal current strength and
duration which did not produce visible motor responses (e.g. whisker
or eyelid twitching) had no effect on plasma levels of β-END-LI
(Fig. 23). The results from these limited experiments might suggest
that raphe serotonin neurons exert little, if any, influence over the
secretion of pituitary β-END-LI. However, in addition to the fact that
the sample size (n=1-3) is small, several other possible explanations
must be considered.

First, and probably most important, pre-stimulation and control
plasma levels of β-END-LI in rats anesthetized and positioned in the
stereotaxic instrument were almost 10 times greater than those observed
in unanesthetized control animals (3.5 ± 0.2 ng/ml vs. 0.30 ± 0.06 ng/ml;
from Fig. 23 and Table 2, respectively), and, in fact, these elevated
levels of plasma β-END-LI were twice as high as those observed in rats
stressed by immobilization (Fig. 12). Thus, it appears that anesthesia
and surgical stress together provide a maximal stimulus for the release
of pituitary β-END-LI and, therefore, stimulation of raphe neurons is
unable to further increase β-END-LI release.

It is also possible that anesthesia and/or surgical stress may
have prevented or masked the subsequent effects of electrical stimulation.

In support of this hypothesis Trulson and Trulson (1983) have shown that anesthesia alters the responsiveness of DRN neurons to a variety of psychoactive drugs, further underscoring the possible interference that anesthesia may have on raphe serotonergic function. Therefore, if both anesthesia and serotonergic activation stimulate pituitary β-END-LI release through a common mechanism (e.g. activation of serotonin neurons) then the combined effect of both stimuli may not necessarily produce additive effects on pituitary β-END-LI secretion. It may also be possible that some of the analgesic effects of anesthesia may be mediated by enhanced serotonergic activity and that further electrical stimulation of these neurons may effectively inhibit further neuronal firing by a negative feedback mechanism. In support of this, it has been shown that increased availability of serotonin in the synapse can induce a depression in the firing rate of raphe neurons (Aghajanian and Gallager, 1976). The important point to note here is the time frame for inhibition of serotonin firing which presumably occurs after sufficient concentrations of serotonin exist in the synaptic cleft to activate presynaptic serotonin receptors which modulate the synthesis and further release of serotonin from the nerve terminal. This most likely does not occur acutely following electrical stimulation when serotonergic effects on pituitary β-END-LI secretion are most likely to occur. However, anesthesia may elevate serotonin concentrations in the synaptic cleft by initiating serotonergic activation and subsequent electrical stimulation may further increase synaptic serotonin levels resulting in an inhibition of serotonin neuronal firing. Clearly, a better model for studying the effects of raphe electrical stimulation on pituitary secretion of a hormone like β-endorphin which is extremely responsive to physical and environmental stimuli

or manipulations should include unanesthetized, unrestrained animals with chronically implanted electrodes for electrical stimulation and indwelling catheters for blood collection.

INVESTIGATION OF THE NEURAL BASIS FOR STIMULATED RELEASE
OF PITUITARY β-END-LI

The observation that physical stimuli, especially those result-ing in pain, evoke a tremendous release of pituitary β-END-LI suggests that pituitary β-END-LI may function in pain and stress adaptation. Hence, investigating the neural basis for stimulated pituitary β-END-LI release may be of importance in providing insights into the physiologic functions of pituitary and blood-borne β-endorphin.

Physical immobilization was found to be a potent stimulus for pituitary β-END-LI release. Following 30 min of restraint, plasma β-END-LI levels rose 12-fold (Fig. 12). Since the basal secretion of β-END-LI appears to be difficult to depress in vivo, the stimulated secretion of β-END-LI may provide a good model for examining the effects of serotonergic manipulation thought to inhibit pituitary β-endorphin release. Accordingly, five and ten days treatment with 5,7-DHT, a sero-tonin neurotoxin which by reducing hypothalamic serotonin content by 75% and 77%, respectively, probably significantly reduced serotonergic func-tion, was shown to reduce the stress-induced release of pituitary β-END-LI (Fig. 12). These findings suggest that serotonin neurons are involved in the stress-induced release of pituitary β-endorphin and are consistent with the published findings of Bruni et al. (1982) in which they showed that fluoxetine treatment potentiated the plasma β-END-LI response to im-mobilization stress. Since similar treatments which abolish serotonergic

function have been reported to reduce opiate and stimulation-produced analgesia, it is possible that this effect may be due, at least in part, to a decrease in serotonin-stimulated pituitary β-END-LI release.

5,7-DHT treatment at a dose of 75 μg did not significantly influence resting levels of plasma β-END-LI in non-stressed animals (Fig. 12). This finding is consistent with the other pharmacological data where serotonin receptor antagonists were unable to depress basal levels of circulating β-END-LI. However, a higher dose (200 μg) of 5,7-DHT was found to reduce resting levels of plasma β-END-LI by 44% as well as significantly reduce hypothalamic serotonin content (Fig. 13). It should be noted that in this latter experiment animals treated with 5,7-DHT for 10 days experienced significant weight loss (20% weight loss compared to control animals) and this effect may have contributed to the observed decrease in basal levels of β-END-LI.

Ether exposure evoked a more moderate release of pituitary β-END-LI as compared to immobilization stress (2.8-fold vs. 12-fold) and thus provides a submaximal stimulus for pituitary β-END-LI release which may be potentiated by the administration of serotonergic drugs known to stimulate β-END-LI release. Fluoxetine can be thought of as a drug which enhances endogenous serotonergic tone by indirectly facilitating serotonergic neurotransmission. If serotonin neurons are involved in the stress-induced release of pituitary β-END-LI, then fluoxetine treatment would enhance this effect and further increase the ether-induced release of pituitary β-END-LI. Animals treated with fluoxetine and then exposed to ether did have significantly higher plasma levels of β-END-LI than animals exposed to ether alone but not fluoxetine alone (Fig. 14). Thus, fluoxetine treatment, at the dose tested, does not appear to potentiate the ether-

induced release of pituitary β-END-LI. One explanation for this obser-
vation is that both ether stress and fluoxetine treatment may be activ-
ating a common serotonergic pathway to stimulate pituitary β-END-LI
secretion thereby preventing an additive effect. Conversely, it may act-
ually appear that fluoxetine treatment blunts that response to ether
stress. These interpretations cannot be resolved on the basis of the
present experiments alone.

The observation that 5,7-DHT treatment significantly attenuated
immobilization stress-induced β-END-LI release, and the reported findings
that immobilization and other forms of stress increase hypothalamic and
whole brain serotonin neuronal activity as reflected by increases in
serotonin metabolism (Mueller et al., 1976; Thierry et al., 1968) suggest
that serotonin neurons are involved in the release of pituitary β-END-LI
in response to immobilization stress. Participation of serotonin neurons
in ether stress-induced pituitary β-END-LI is, however, less clear. Fur-
thermore, is has been shown that centrally administered β-endorphin in-
creases serotonin metabolism (Van Loon and De Souza, 1978). Therefore, it
is possible that the enhanced serotonergic metabolism observed during
stress may result as a consequence of stimulated pituitary β-endorphin
secretion. However, the observation that 5,7-DHT treatment reduced the
stress-induced release of β-END-LI indicates that serotonergic activation
is necessary for the stres-induced release of β-END-LI and most likely
preceeds this event.

The precise role which brain serotonergic neurons and pituitary
β-END-LI secretion play in the physiological response to stress have yet
to be determined. Recent observations suggest that the behavioral
analgesia that results as a consequence of stress requires serotonin

neurotransmission (Bhattacharya et al., 1978) and opiate receptor ligands (Lewis et al., 1980) of pituitary origin (Amir and Amit, 1979). The present findings that brain serotonin neurons are involved in the stress-induced release of pituitary β-END-LI further suggest that stress-induced analgesia may result as a consequence of serotonergic stimulation of pituitary β-END-LI secretion.

DETERMINATION OF THE PITUITARY SOURCE (AL vs. IL) FOR CIRCULATING β-END-LI

In vitro AL and IL cells secrete distinctive forms of β-END-LI which most likely reflect the differential secretion of these peptides from the AL and IL in vivo. Cultured AL cells secrete β-LPH-sized and β-END$_{1-31}$-sized immunoreactivity whereas the IL secretes predominantly the smaller and acetylated forms of β-END$_{1-31}$ and no β-LPH (Liotta et al., 1978; Eipper and Mains, 1981; Smyth and Zakarian, 1980). Since only cultured AL cells secrete appreciable amounts of the analgesic form of β-END-LI (β-END$_{1-31}$), determination of the molecular forms of β-END-LI secreted in response to serotonergic stimulation is of particular interest in evaluating the role of serotonin neurons and pituitary β-END-LI in current models of endogenous analgesia. To determine the pituitary source (AL vs IL) for blood-borne β-END-LI, β-LPH-sized and β-END-sized immunoreactivity were resolved on the basis of molecular weight using gel filtration chromatography and identified by comparing their position in the elution profile with that of known standards. The presence of β-LPH-sized immunoreactivity in plasma was interpreted to reflect AL secretion of β-END-LI in vivo, since only cultured AL cells secrete β-LPH (Fig. 15).

A comparison of the gel filtration elution profiles from control

and drug-treated animals revealed that tryptophan, 5-HTP, fluoxetine and quipazine, drugs which enhance serotonergic neurotransmission, predominantly increased the form of plasma β-END-LI corresponding to β-LPH in molecular size (Fig. 16 and 17). Virtually all of the rise in plasma β-END-LI following tryptophan administration was due to β-LPH-sized immunoreactivity. 5-HTP, fluoxetine and quipazine also increased to a lesser extent the peak of immunoreactivity resembling β-END in molecular size. These results suggest that serotonin neurons predominantly stimulate the release of β-END-LI from AL corticotrophs. These conclusions are further supported by the recent findings of Petraglia et al. (1982) in which serotonin agonist drugs were shown to deplete AL but not affect NIL β-END-LI content and elevate plasma β-LPH and β-END levels.

The observation that serotonergic drugs predominantly release a form of β-END-LI resembling β-LPH in molecular size raises questions about the physiologic importance of circulating β-LPH. To date, the functions for plasma β-LPH are unclear. Some evidence suggests that β-LPH may play a role in aldosterone regulation (Matsuoka et al., 1980) and lipid metabolism (Li, 1964). However, β-LPH does not appear to bind opiate receptors (Cox et al., 1976) and does not possess analgesic properties. In addition, little, if any, conversion of β-LPH to β-END occurs in blood (Aronin et al., 1981; Houghten et al., 1980) suggesting that β-LPH in blood most likely does not function as a precusor pool for circulating β-endorphin. Although it has not yet been demonstrated, it is possible that β-LPH may act to permit or modulate β-endorphin's bioactivity. Of course, a role for β-LPH unrelated to β-endorphin and analgesia is also possible.

Since both β-LPH and β-END immunoreactivity are secreted *in vitro*

from AL cells, then it is expected that some β-END$_{1-31}$ is released con-
comitant with the observed rise in β-LPH and this is evidenced by the ob-
served increase in the β-END peak of immunoreactivity. The total concen-
trations of β-END$_{1-31}$ in plasma are probably too small to produce an anal-
gesic effect. However, the amount of β-END$_{1-31}$ entering the brain from
the pituitary is unknown and may significantly contribute to CNS mechan-
isms of analgesia. It is also possible that other physiologic functions
unrelated to analgesia and presently unknown may be likely for serotonin-
induced β-END-LI release.

The molecular forms of β-END-LI in plasma most likely represent
the overall release of β-END-LI from both the AL and IL in vivo. Based
on the in vitro release patterns, one would expect plasma responses of
similar magnitudes in the release of β-LPH and β-END since these two forms
appear to be secreted about equally from AL cultures (Fig. 15). The obser-
vation that plasma from animals treated with serotonergic drugs contained
greater amounts of β-LPH than β-END suggests that the secretory patterns
of cells in culture may not reflect exactly the proportions in which
these peptides are secreted in vivo. In any event, it is evident that
the AL is the principle origin of the β-END-LI released in response to ser-
otonergic drugs suggesting that the immunoreactivity resembling β-END in
molecular size most probably is the analgesic form secreted from the AL.

It remains to be determined to what extent experimentally-induced
changes in the processing of the β-END precursor molecule or the clearance
rates for the different forms of plasma β-END-LI may have contributed to
the observed shifts in the chromatographic profiles of plasma β-END-LI.
β-LPH and β-END$_{1-31}$ have been shown to be relatively stable in blood
(Houghten et al., 1980; Aronin et al., 1981) and little, if any, conver-

sion of β-LPH to β-END has been shown to occur under the blood collect-
ion, storage and handling procedures employed here (unpublished labora-
tory findings). Blood was collected into plastic tubes containing baci-
tracin, a protease inhibitor, and EDTA, a calcium chelator, and kept on
ice until plasma was separated by centrifugation (at 4°C). Plasmas
were stored at −70°C which further insures inactivation of all metabolic
processes. In addition, β-END-LI has been shown to be stable in the
weak acid eluent (0.1 N acetic acid) used in the chromatographic separ-
ations. These procedures assure that the observed ratios of β-LPH/β-END
accurately reflect the ratio present in blood at the time of blood col-
lection.

The chromatographic profiles provide useful information about
the ratio or relative amounts of β-LPH and β-END in plasma pooled from
each treatment group. Plasma was necessarily pooled in order to achieve
adequate amounts of total β-END-LI to be resolved by gel filtration chro-
matography. The plasma elution profiles, therefore, provide qualitative
rather than strictly quantitative information about the molecular forms
of plasma β-END-LI. Nevertheless, the results do lend themselves to logi-
cal interpretation as elution profiles of plasma from animals treated
with the same drug in separate experiments consistently yield similar
results.

The elution profiles of plasma from control animals in various
and separate experiments shows that the β-LPH peak of immunoreactivity
comprises 20%-50% of total β-END-LI. This finding plus the observation
that dexamethasone, a selective antagonist of AL corticotroph secretion,
reduced circulating levels of β-END-LI to below the limit of assay detec-
tion in control animals (Fig. 21) suggest that the AL is the principle

source for the basal (spontaneous) release of β-END-LI. It is likely that the IL may also contribute to the basal secretion of β-END-LI since α-MSH, a peptide co-secreted with β-END-LI from the IL, is present in normal rat blood (Usategui et al., 1976). However, evidence for the existence of the smaller and acetylated forms of $β-END_{1-31}$ in normal rat blood remains unclear and questionable suggesting that the basal secretion of β-END-LI from the IL may be negligible. Interestingly, the IL contains over 90% of the total β-END-LI present in the pituitary gland (Mueller, 1980). Therefore, it is surprising to find (and difficult to explain) that this lobe contributes so little, if at all, to the basal secretion of β-END-LI. This observation raises the question of the importance of the IL in the rat and further supports the view that this portion of the pituitary is most probably vestigal.

The observation that cinanserin and 5,7-DHT, a serotonin receptor blocker and neurotoxin, respectively, reduced both β-LPH and β-END-sized immunoreactivity in response to serotonergic stimulation and stress, respectively (Fig. 18 and 19), further supports the hypothesis that the rise in β-LPH and β-END is mediated through a serotonergic mechanism. In addition, these findings further substantiate that β-END-sized immunoreactivity ($β-END_{1-31}$) is released from the AL along with β-LPH in response to serotonergic stimulation and stress. Both cinanserin and 5,7-DHT treatments did not alter the total levels or ratios of the molecular forms of β-END-LI in control (non-stressed) animals indicating that these treatments were unable to reduce the basal secretion of pituitary β-END-LI.

As observed with the administration of serotonergic drugs, immobilization and ether stress also increased total levels of plasma β-END-

LI. Elution profiles of plasma from animals stressed by immobilization or ether demonstrate an increase in both forms of immunoreactivity with a relatively greater rise in β-LPH than β-END (Fig. 19 and 20). This suggests that stress also activates AL corticotroph secretion of β-END-LI. This conclusion is further supported by the well-known fact that stress elevates corticotrophic ACTH release in rats (Guillemin et al., 1977). A dramatic rise in β-END-sized immunoreactivity was also observed in the plasma of these animals suggesting that a significant portion of total plasma β-END-LI may be derived from the IL in response to stress. In support of this view, it has been reported that the selective destruction of the NIL attenuates the stress-induced release of β-END-LI in rats (Przewlocki et al., 1982). Furthermore, it has been reported that α-MSH a secretory product released along with β-END peptides from IL melano-trophs (Mains and Eipper, 1979), is elevated in plasma of stressed rats (Mialhe and Briaud, 1977; Tilders et al., 1982). In addition, another possible explanation for the dramatic rise in β-END-sized immunoreactivity is that stress may trigger the release of factor(s) into blood which may act to promote the further cleavage of circulating β-LPH to β-END in vivo. The release of such factor(s) into blood in response to stress is presently unknown and may be of interest to investigate.

In summary, serotonergic drugs and stress predominantly influence the release of a form of β-END-LI known only to be released from cultured AL cells suggesting that the rise in plasma β-END-LI in response to serotonergic stimulation or stress originates from AL corticotrophs. Accordingly, the pronounced rises in plasma β-END-LI resembling β-LPH and β-END evoked by serotonergic drugs and stress are probably mediated through serotonin neurons which regulate the release of hypothalamic

CRF, the hypothalamic hormone which regulates AL corticotrophs and hence, β-END-LI and ACTH. Serotonin has been reported to stimulate the secretion of CRF from hypothalamic tissue in vitro (Buckingham and Hodges, 1979), and CRF has been shown to be a potent releaser of β-LPH-sized immunoreactivity from cultured AL cells (Vale et al., 1978) and to elevate circulating levels of β-END-LI in vivo (Rivier et al., 1982). These conclusions are further supported by the observation that dexamethasone, a selective antagonist of CRF-stimulated corticotropin secretion in vitro (Vale et al., 1978, 1979, 1983), preferentially reduced circulating levels of β-END-LI corresponding to β-LPH under basal conditions and following serotonergic activation (Fig. 21). Although dexamethasone lowered circulating levels of β-LPH-like immunoreactivity to below the limit of assay detection in control animals, dexamethasone was unable to completely prevent the release of β-LPH in fluoxetine-treated animals at a dose of 50 µg/kg. In addition, dexamethasone has been reported to reduce the stress-induced release of pituitary β-END-LI (Guillemin et al., 1977) and to attenuate stress-induced analgesia (Lewis et al., 1980). This further supports the possibility that serotonergic activation and stress evoke the release of an analgesic form of β-endorphin, β-END$_{1-31}$, from AL corticotrophs. If this is the case, then these findings strengthen the hypothesis that serotonergic stimulation of pituitary β-END$_{1-31}$ release may represent another means by which serotonin neurons and endorphins interact together in endogenous mechanisms of analgesia. Furthermore, if plasma levels of β-END$_{1-31}$ are preferentially elevated in response to serotonin and stress, then this suggests that β-END$_{1-31}$ may have a physiologic function in stress analgesia since analgesia is observed during stress and enhanced sero-

tonergic neurotransmission.

THE EFFECTS OF SEROTONIN HCL AND CRF ON THE RELEASE OF β-END-LI FROM CULTURED ANTERIOR AND INTERMEDIATE LOBE CELLS

The regulation of AL and IL β-END-LI release, like the biosynthetic processing of the POMC precursor, appears to be different for these two lobes of the pituitary. AL β-END-LI appears to share common humoral regulatory mechanisms with ACTH whereas IL β-END-LI is most likely regulated by the neurons which directly innervate this lobe. Based on current knowledge of the anatomy and physiology of the pituitary gland, it is presumed that serotonergic regulation of AL β-END-LI is mediated through its influence on hypothalamic β-END releasing factor(s) rather than by a direct effect on pituitary corticotrophs.

In vitro, it was shown that serotonin had no direct effect on the spontaneous release of β-END-LI from cultured AL cells at concentrations which fall within a physiologic range (10^{-9}-10^{-7}M). At a higher concentration (10^{-6}M), serotonin did evoke significant release of β-END-LI by AL cells. However, this response is likely to be non-physiologic since CRF, at a concentration of 10^{-9}M (one thousandth of that for serotonin) was capable of stimulating AL β-END-LI release. The stimulatory effects of CRF on AL β-END-LI release occurred over the range of 10^{-8}M-10^{-9}M and confirm previous findings from other laboratories in which CRF was demonstrated to be a potent stimulator of β-END-LI release in vitro (Vale et al., 1983) and in vivo (Rivier et al., 1982). Together, these results confirm the hypothesis that serotonergic regulation of AL β-END-LI release is mediated by a hypothalamic mechanism rather than by a direct effect on AL corticotrophs.

It is likely that serotonergic control over AL β-END-LI release is mediated by hypothalamic CRF since serotonin has been shown to stimulate hypothalamic CRF release in vitro (Buckingham and Hodges, 1979; Jones et al., 1976) and in vivo (Fuller, 1981). In addition, ascending midbrain serotonergic pathways are known to innervate areas which have recently been identified immunohistochemically to contain CRF cell bodies (Olschowka et al., 1982). Furthermore, the observation that dexamethasone, a specific antagonist of CRF-mediated corticotropin secretion, attenuated the serotonergic-induced rise in plasma β-END-LI (Fig. 21) indicates that at least a part of serotonin-stimulated β-END-LI release involves CRF. Other weaker secretagogues of AL β-END-LI release (vasopressin, oxytocin, catecholamines, angiotensin II) have been shown to potentiate CRF's action in vitro (Vale et al., 1983) and in vivo (Rivier and Vale, 1983) suggesting that CRF interacts with other modulators in the regulation of pituitary β-END-LI. Thus, it is possible that serotonin's control over adenohypophysial β-END-LI release is mediated through its influence on CRF, the major physiologic releaser of β-END-LI, and/ or other substances which modulate CRF action.

The recent report that a specific high affinity serotonin uptake system exists in rat anterior pituitary tissue (Johns et al., 1982) raises the possibility of a direct effect of serotonin on the pituitary as was observed with the highest concentration of serotonin on cultured AL cells (Fig. 22). However, it is possible (although not proven) that these serotonin binding sites are located on serotonin-rich mast cells present in pituitary vasculature (Bjorklund et al., 1973) or on pituitary blood vessels since serotonin receptors do exist on peripheral blood vessels (Westlund and Childs 1982). The influence that these vascular serotonin

receptors may have on corticotropic β-END-LI release is uncertain and dubious.

The observation that neither CRF or serotonin were able to alter the spontaneous release of β-END-LI from cultured NIL cells (Fig. 22) suggests that if CRF and serotonin are involved in the regulation of NIL β-END-LI then this regulation is most likely mediated by the dopaminergic neurons directly innervating the IL. In addition, it may be possible that not high enough concentrations of CRF and serotonin were used to evoke the release of β-END-LI. Recent reports show that CRF is a weak releaser of NIL β-END-LI (Vale et al., 1983) and α-MSH (Meunier et al., 1982; Proux-Ferland et al., 1982) in vitro. However, it is unknown whether CRF regulation of pars intermedia β-END-LI and α-MSH secretion occurs in vivo since considerably higher concentrations of CRF are required to stimulate β-END-LI release from cultured IL than AL cells. Since the IL has little, if any, contact with the hypothalamic-hypophysial portal blood system where CRF and other hypothalamic hormones are released, then CRF regulation of IL melanotrophs would most likely be mediated directly by CRF neurons. Presently, it is unknown whether CRF nerve terminals innervate the pars intermedia or any other portion of the pituitary gland.

Interestingly, the IL is directly innervated by serotonin neurons. Mezey et al. (1983) have recently detected serotonin-containing nerve fibers and terminals in the rat pituitary intermediate lobe by light and electron microscopic immunohistochemistry. Their findings further indicate that the origin of these serotonergic nerve fibers is from the CNS suggesting that these neurons may serve to regulate IL function. However, others have failed to show that serotonin can modify the release

of IL β-END-LI in vitro (Vale et al., 1979). Together with the present observations, the findings suggest that although serotonin nerve fibers innervate the IL, these neurons apparently exert little effect on the release of β-END-LI from this lobe in vivo. Conversely, catecholaminergic neurons densely innervate the pars intermedia (Howe, 1973) and are known to directly influence IL β-END-LI (Pettibone and Mueller, 1982) and α-MSH (Cote et al., 1982) release in vitro. These and other in vivo data indicate that hypothalamic dopaminergic and noradrenergic neurons exert direct and reciprocal control over α-MSH and β-END-LI secretion from the IL (Vale et al., 1979; Vermes et al., 1980; Tilders and Smelik, 1977; Farah et al., 1982). Together these findings indicate that catecholaminergic neurons are probably physiologically important regulators of IL β-endorphin release. The present observations that neither CRF nor serotonin significantly altered IL β-END-LI release in vitro at the concentrations applied suggest that these neurons probably are not physiologically important regulators of IL β-END-LI release in vivo. Consistent with this is the observation that serotonergic drugs which elevate plasma levels of β-END-LI, lower AL but not NIL content of β-END-LI (Petraglia et al., 1982).

In summary, serotonergic control of AL β-END-LI release is most likely mediated via serotonergic control over the release of β-endorphin releasing factor(s), probably CRF, into hypophysial portal blood. Conversely, CRF and serotonin neurons may participate in the regulation of IL β-END-LI by modulating the activity of noradrenergic and dopaminergic neurons known to directly influence the release of β-END-LI from this lobe.

CONCLUSIONS

The present data indicate that serotonin neurons are involved in the basal and stress-induced release of pituitary β-endorphin. The observation that serotonin neurons preferentially stimulate the release of β-END-LI from the anterior lobe which is the predominant pituitary source for the analgesic form of β-endorphin further suggest that the analgesia associated with serotonergic activation and stress may be mediated, at least in parc, by the release of pituitary β-endorphin. These findings provide evidence for the existence of a functional relationship between serotonin neurons and behavioral analgesia involving pituitary β-endorphin.

A summary diagram illustrating the possible relationships between brain serotonin neurons and pituitary opiate peptides participating in endogenous mechanisms of analgesia is shown in Figure 24. Descending serotonin neurons (from the Nucleus Raphe Magnus) are known to modulate nociception at the spinal cord level by inhibiting pain transmitting neurons (primary afferent substance P-containing neurons) in the dorsal horn. This inhibition is most likely mediated through an enkephalinergic interneuron. The pain-transmitting neurons project to supraspinal levels and indirectly, via the N. Reticularis gigantocellularis, contact the cells of the descending analgesia system in the periaqueductal gray and N. Raphe Magnus thus establishing a negative-feedback loop. Activation of the primary afferent pain-transmitting neurons by noxious stimulation (detected by peripheral pain receptor) turns on the descending pain-suppression system which effectively blocks further pain transmission at the spinal cord level. Ascending serotonin neurons (most likely the

Dorsal and Median Raphe Nuclei) may modulate nociception by stimulating the release of opiate active $\beta\text{-END}_{1-31}$ from the anterior lobe of the pituitary gland into blood. The release of anterior lobe $\beta\text{-END}_{1-31}$ is most likely mediated through serotonergic regulation of hypothalamic CRF, a known potent physiologic stimulator of ACTH and β-END. Pituitary β-END may enter the CNS either through retrograde flow up the hypophysial portal vessels into the hypothalamus or by penetration of less permeable sites of the blood-brain barrier. Within the CNS, β-END may bind to opiate receptors located in the periaqueductal gray, an area known to produce analgesia upon electrical stimulation or the administration of opiate peptides, and/or bind opiate receptors which would activate serotonergic neurotranmission in the N. Raphe Magnus and thereby facilitate pain suppression at the spinal cord level. Binding of β-END to opiate receptors in the limbic lobe may alter the perception of pain. Outside the CNS, blood-borne $\beta\text{-END}_{1-31}$ may desensitize peripheral pain receptors and further modulate nociception. Circulating β-END-LI may serve an important function in areas unrelated to analgesia. Some of these functions may include its action on the adrenal gland to stimulate corticosterone release, on gastrointestinal tissues to modulate GI motility and pancreatic release of glucagon, and on cardiovascular tissues to regulate peripheral cardiovascular function. In addition, blood-borne β-END may play a role in the immune response and thus provide a functional link between the endorine and immune systems.

In summary, serotonergic regulation of anterior lobe β-endorphin$_{1-31}$ release may be an important part of an endogenous analgesia mechanism involving CNS and peripheral sites of action for circulating β-endorphin$_{1-31}$ which contribute to the animal's global response to

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Figure 24. A summary diagram illustrating the possible relationships between brain serotonin neurons and pituitary opiate peptides in endogenous mechanisms of analgesia. Available evidence indicates that serotonin neurons modulate nociception via ascending and descending pathways. Descending serotonin neurons inhibit the transmission of pain (initiated by peripheral pain receptors) at the dorsal horn of the spinal cord. Ascending serotonin neurons (and stress) may modulate nociception by stimulating the release of opiate-active β-END from the anterior lobe of the pituitary into blood. The release of anterior lobe β-END is most likely mediated through serotonergic regulation of hypothalamic corticotropin-releasing factor (CRF), a potent physiologic β-END releasing factor. Blood-borne β-END may enter the brain to: (1) bind to opiate receptors located in areas involved in the development of analgesia and (2) enhance descending serotonergic neurotransmission and thereby facilitate pain suppression at the spinal cord level. In addition, blood-borne β-END may desensitize peripheral pain receptors. Other functions for circulating β-END unrelated to analgesia may include its action on the adrenal gland, GI tract and cardiovascular tissues, as well as the pancreas and immune system. Physiologic functions for blood-borne β-LPH, released together with β-END, are presently unknown. Adrenal glucocorticoids feedback negatively to regulate pituitary β-END and ACTH secretion.

Figure 24

pain and stress. Other unknown physiologic functions of pituitary β-END-LI which are unrelated to analgesia or stress adaptation but involve serotonin neurons may also exist. Of particular interest are the possible physiological functions for circulating β-LPH which remain to be determined. The observation that serotonergic and stress stimuli evoke a dramatic release of β-LPH suggests that this hormone may serve an important physiologic role within the body.

APPENDIX I

LIST OF ABBREVIATIONS

Abbreviation	Term
ACTH	adrenocorticotropin
ADH	aldehyde dehydrogenase
AL	anterior lobe of pituitary (pars distalis)
β-END	β-endorphin
β-END-LI	β-endorphin-like immunoreactivity
β-LPH	β-lipotropin
CLIP	corticotropin-like intermediate lobe peptide
CNS	central nervous system
CRF	corticotropin-releasing factor
5,7-DHT	5,7-dihydroxytryptamine; serotonin neurotoxin
DRN	dorsal raphe nucleus
5-HIAA	5-hydroxyindoleacetic acid; serotonin metabolite
5-HT	5-hydroxytryptamine; serotonin
5-HTP	5-hydroxtryptophan; serotonin precursor
icv	intra-cerebro-ventricular administration
IL	intermediate lobe of pituitary; pars intermedia
ip	intraperitoneal administration
leu-enkephalin	leucine-enkephalin
L-AADC	L-aromatic amino acid decarboxylase
MAO	monoamine oxidase
met-enkephalin	methionine-enkephalin

Abbreviation	Term
MRN	Median raphe nucleus
MSH	melanocyte-stimulating hormone
NIL	neurointermediate lobe of pituitary (pars intermedia plus pars nervosa)
NRM	Nucleus raphe magnus
OA	opiate analgesia; morphine analgesia
PAG	periaqueductal gray area
PCPA	para-chlorophenylalanine; serotonin biosynthesis inhibitor
POMC	pro-opiomelanocortin; 31K β-endorphin precursor
RIA	radioimmunoassay
SPA	stimulation-produced analgesia
TOH	tryptophan hydroxylase
TRYP	tryptophan

APPENDIX II

β-ENDORPHIN RADIOIMMUNOASSAY

REAGENTS AND MATERIALS

Assay buffer: 0.05M phosphate buffer containing 0.05% bovine serum

albumin (BSA),   0.02% sodium azide and 5 mg% bacitracin; pH 7.3

Dissolve in four liters of distilled water the following:

6.62 g monobasic sodium phosphate ($NaH_2PO_4-H_2O$; Fisher Scientific
Co., Fairlawn, NJ)
21.58 g dibasic sodium phosphate (Na_2HPO_4; Fisher Scientific Co.)
200 mg bacitracin (Sigma)
0.80 g sodium azide (Sigma)
2.0 g bovine serum albumin, fraction V (Sigma)

β-Endorphin antibody (C-55):   initial concentration of 1:20,000 in assay

buffer; 100 µl pipetted into assay reaction mixture to final volume of

500 µl.   Final antibody concentration of 1:100,000.

Radiolabeled β-endorphin):   15,000-20,000 counts per minute per

100 µl assay buffer.

Heat-inactivated horse serum (HIHS; GIBCO) or plasma from hypophysectomized

rats (Hormone Assay Labs, Chicago, IL):   50-100 µl added to standard curve

and each non-plasma sample tube to control for plasma binding effects.

Charcoal:BSA (1:0.1):   0.55 g/100 µl 0.05M phosphate buffer

Phosphate buffer: 6.62 g monobasic sodium phosphate and 21.58 g
dibasic sodium phosphate dissolved in 4 liters of distilled water

Charcoal/BSA mixture: 10 g activated charcoal U.S.P. (Mallinckrodt,
Inc., St. Louis, MO) and 1 g BSA mixed together

Test tubes: 12x75 mm disposable borosilicate glass culture tubes

(Scientific Products, McGaw Park, IL)

PROCEDURE FOR STANDARD CURVE

Camel β-endorphin$_{1-31}$ (Peninsula Labs, San Carlos, CA) dissolved in assay

buffer to concentrations of 6 pg, 10 pg, 30 pg, 60 pg, 100 pg, 300 pg,

600 pg and 1 ng standard per 100 µl assay buffer.

1. Total counts tubes (in duplicate) contain 100 µl tracer

2. Nonspecific binding tubes (in duplicate) contain 100 µl tracer plus 350 µl assay buffer plus 50 µl HIHS

3. Specific binding tubes (in duplicate) contain 100 µl antibody, 100 µl tracer, 250 µl assay buffer and 50 µl HIHS

4. Standard curve tubes (in duplicate) contain 100 µl of β-endorphin standard, 150 µl assay buffer, 100 µl antibody, 100 µl tracer and 50 µl HIHS

PROCEDURE FOR SAMPLE TUBES

1. 50 µl and 100 µl samples or 100 µl and 200 µl samples pipetted into test tubes in duplicate

2. 50 µl of HIHS or 100 µl plasma from hypophysectomized rats added to non-plasma sample tubes

3. Assay buffer added to bring total reaction mixture volume to 500 µl for all sample tubes

4. 100 µl antibody added to each tube

5. 100 µl tracer added to each tube

6. All assay test tubes (samples and standard curve) vortexed and incubated (see below).

INCUBATION

1. Assays containing plasma samples incubated 72 hrs at 4°C

2. Assays containing chromatography elution profiles, tissues or culture media samples incubated at room temperature (37°C) for 24 hrs.

PROCEDURE FOR SEPARATION OF FREE FROM ANTIBODY-BOUND RADIOACTIVITY

1. 1 ml of charcoal/BSA mixture added to all test tubes except total counts tubes and vortexed

2. Test tubes allowed to sit for 30 min and then centrifuged for 30 min at 2500 RPM

3. Supernatants decanted into test tubes and ^{125}I radioactivity (gamma wave emittance) measured on a Gamma counter (Searle Analytic Inc.)

CALCULATIONS

Standard curve plotted (pg β-END vs. counts) on semi-log paper.

Amount of β-END in sample tubes determined from standard curve and corrected to ng β-END-LI/ml for plasma samples; ng β-END-LI/fraction for elution profiles; ng β-END-LI/plate for culture media, and ng β-END-LI per mg protein for tissue content.

APPENDIX III

SPECTROFLUOROMETRIC SEROTONIN ASSAY

REAGENTS

Acid butanol – 1 liter butanol plus 0.85 ml concentrated hydrochloric acid (HCl)

0.5M Phosphate buffer – titrate dibasic 0.5M phosphate with monobasic 0.5M phosphate to pH 7.5

1% Cysteine – 0.1 g L-cysteine (Sigma) dissolved in 10 ml 0.1N HCl

OPT solution – 4.0 mg O-phthaldialdehyde (A grade, Calbiochem, San Diego, CA) dissolved in 100 ml concentrated HCl

Stock serotonin standard – 43.97 mg serotonin creatinine sulfate (Sigma) in 100 ml of 0.1 N HCl (20 mg free base/100 ml), store at 4°C for up to three weeks

PROCEDURE FOR BRAIN SAMPLES

1. Homogenize brain (about 1.7 g tissue) in 7.0 ml acid butanol and keep on ice

2. Centrifuge homogenate at 17,000 RPM for 20 min at 4°C

3. Transfer 3.0 ml of supernatant into a glass screw cap test test containing 2.5 ml of 0.1N HCl and 6.0 ml heptane

4. Shake for 10 min

5. Centrifuge at maximum speed in an International Clinical Centrifuge (IEC, Needham Hts., MA) for 5 min

6. Draw off organic phase by suction and transfer 1.ml aqueous phase into a glass screw cap test test containing 100 μl of 1% cysteine

7. Add 2.0 ml OPT solution, mix and cap

8. Heat for 10 min in 100°C water bath with lid on

9. Cool to room temperature and read sample fluorescence at 355 nm

excitation and 470 nm emission

PROCEDURE FOR HYPOTHALAMIC SAMPLES

1. Homogenize hypothalamus in 2.0 ml acid butanol

2. Rinse with 1.0 ml acid butanol and combine with initial homogenate

3. Centrifuge at 17,000 RPM for 20 min at 4°C

4. Transfer supernatant (2.0 ml) to a glass screw cap test tube containing 2.5 ml of 0.1N HCl and 6.0 ml heptane

   Procede to step 4 in the procedure for brain samples

PROCEDURE FOR STANDARDS

1. Dilute serotonin stock solution 1:100 with 0.1 N HCl. Final concentration of 'working' standard solution is 200 ng/100 μl

2. Serotonin standard curve: place 0 μl, 25 μl, 50 μl and 100 μl of working standards (in duplicate) into glass screw cap test tubes containing 0.1 ml 1% cysteine and 1.0 ml, 0.975 ml, 0.950 ml and 0.900 ml 0.1N HCl. The standard tubes will contain 0 ng, 50 ng, 100 ng and 200 ng serotonin, respectively. Begin at step 7 in the procedure for brain samples

3. Internal standards – to determine percent recovery for brain extraction procedure. Place in duplicate, 0 μl, 100 μl, and 200 μl of working serotonin standard (0 ng, 200 ng and 400 ng serotonin) into glass screw cap test tubes containing 3.0 ml acid butanol, 6.0 ml heptane and 2.5 ml, 2.4 ml and 2.3 ml 0.1N HCl, respectively. Begin at step 4 in the procedure for brain samples

4. Internal standards – to determine percent recovery for hypothalamus extraction procedure. Place, in duplicate, 0 μl, 50 μl and 100 μl of working serotonin standard (0 ng, 100 ng and 200 ng) in glass screw cap test tubes containing 3.0 ml acid butanol, 6.0 ml heptane and

1.5 ml, 1.45 ml anad 1.4 ml 0.1N HCl, respectively. Begin at step
4 in the procedure for brain samples

APPENDIX IV

CURRICULUM VITAE

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                                    Neuropharmacology
                                    Peptide Hormones

Published Abstracts:

1.   Sapun DI, Farah JM Jr., Mueller GP, Evidence for serotonergic stim-
      ulation of pituitary beta-endorphin (B-END) release in rats. 62th
      Annual Endocrine Society Abstracts p. 217 (572), 1980.

2.   Farah JM Jr., Sapun DI, Mueller GP, Dopaminergic inhibitory control
      of pituitary beta-endorphin and prolactin secretion in the rat.
      62th Annual Endocrine Society Abstracts p. 104 (119), 1980.

3.   Sapun DI, Farah JM Jr, Premen AJ, Mueller GP, Regulation of pitui-
      tary beta-endorphin secretion: Serotonergic stimulation and dopa-

Cirriculum Vitae
Diana Sapun Malcolm

Published Abstracts Contd.

minergic inhibition. Federation Proceedings 40:305 (408), 1981.

4. Farah JM Jr, Sapun DI, Mueller GP, The secretion of pituitary beta-endorphin-like immunoreactivity is regulated independently by dopamine and glucocorticoids in the rat. Society for Neurosciences Abstracts p. 182 (397), 1981.

5. Mueller GP, Pettibone DJ, Deeter WT, Sapun DI, Farah JM Jr, In vivo release of beta-endorphin immunoreactivity by rat pars distalis: inhibition by glucocorticoids. 63th Annual Endocrine Society Abstracts p. 182 (397), 1981.

6. Sapun-Malcolm D, Farah JM Jr, Mueller GP, Evidence for serotonergic regulation of pituitary beta-endorphin release from the anterior lobe. Physiologist 25:261 (36.2), 1982.

7. Farah JM Jr, Sapun-Malcolm D, Mueller GP, Dopaminergic mechanisms inhibit the release of immunoreactive beta-endorphin from both the anterior and intermediate lobes of the rat pituitary. Physiologist 25:261 (36.1), 1982.

8. Mueller GP, Pettibone DJ, Meyerhoff J, Sapun-Malcolm D, Microwave irradiation increases brain concentrations of immunoreactive beta-endorphin and alpha-melanotropin but not neurotensin. 64th Annual Endocrine Society Abstracts p. 228 (594), 1982.

9. Sapun-Malcolm D, Farah JM Jr, Mueller GP, Evidence for independent serotonergic and dopaminergic regulation of pituitary beta-endorphin release in rats. 65th Annual Endocrine Society Abstracts p. 91 (42), 1983.

10. Farah JM Jr, Sapun-Malcolm D, Mueller GP, Regulation of pituitary beta-endorphin secretion by dopamine receptor subtypes. 65th Annual Endocrine Society Abstracts, p. 319 (956), 1983.

Published Papers:

1. Sapun DI, Farah JM Jr, Mueller GP, Evidence that a serotonergic mechanism stimulates the secretion of pituitary beta-endorphin-like immunoreactivity in the rat. Endocrinology 109:421 (1981).

2. Farah JM Jr, Sapun-Malcolm D, Mueller GP, Dopaminergic inhibition of pituitary beta-endorphin-like immunoreactivity secretion in the rat. Endocrinology 110:657 (1982).

Curriculum Vitae
Diana Sapun Malcolm

Published Papers Contd.

3. Sapun-Malcolm D, Farah JM Jr, Mueller GP, Evidence for serotoner-
    gic stimulation of pituitary beta-endorphin release: preferential
    release from the anterior lobe in vivo. Life Sciences 33:95 (1983).

4. Farah JM Jr, Sapun-Malcolm D, Mueller GP, Evidence for dopaminergic
    inhibition of pars distalis and pars intermedia secretions of pitui-
    tary beta-endorphin-like immunoreactivity in vivo. (In preparation).

5. Mueller GP, Pettibone DJ, Farah JM Jr, Sapun-Malcolm D, In vivo
    release of beta-endorphin immunoreactivity by rat pars distalis:
    inhibition by glucocorticoids. (Submitted for publication).

6. Farah JM Jr, Sapun-Malcolm D, Mueller GP, The dopaminergic agonist
    LY141865 stimulates pituitary release of immunoreactive beta-end-
    orphin in rats (In preparation).

REFERENCES

Aghajanian GK, and Asher IM 1971 Histochemical fluorescence of
    raphe neurons: selective enhancement by tryptophan.
    Science 172:1159-1161

Aghajanian GK, and Gallager 1976 Inhibition of firing of raphe
    neurons by tryptophan and 5-hydroxytryptophan: blockade by
    inhibiting serotonin synthesis with Ro-4-4602. Neuropharm-
    acology 15:149-156

Akil H, and Mayer DJ 1972 Antagonism of stimulation-produced
    analgesia by p-CPA, a serotonin synthesis inhibitor.
    Brain Res 44:692-697

Akil H, and Liebeskind JC 1975 Monoaminergic mechanisms of stim-
    ulation-produced analgesia. Brain Res 94:279-296

Akil H, Mayer DJ, and Liebeskin JC 1976 Antagonism of stimula-
    tion-produced analgesia by naloxone, a narcotic antagonist.
    Science 191:961-962

Akil H, Ueda Y, Lin HL, and Watson SJ 1981a A sensitive coupled
    HPLC/RIA technique for separation of endorphins: multiple
    forms of β-endorphin in rat pituitary intermediate vs.
    anterior lobe. Neuropharmacology 1:429-446

Akil H, Young E, Watson SJ, and Coy D 1981b Opiate binding pro-
    perties of naturally occuring N- and C-terminus modified
    β-ENDs. Peptides 2:284-292

Amir S, and Amit Z 1978 Endogenous opioid ligands may mediate
    stress-induced changes in the affective properties of pain
    related behavior in rats. Life Sci 23:1143-1152

Amir S, and Amit Z 1979 The pituitary gland mediates acute and
    chronic pain responsiveness in stressed and non-stressed
    rats. Life Sci 24:439-448

Amir S, Brown ZW, and Amit Z 1979 The role of endorphins in
    stress: Evidence and speculations. Neurosci Biobehav Rev
    4:77-86

Aronin N, Wiesen M, Liotta AS, Schussler GC, and Krieger DT 1981
    Comparative metabolic clearance rates of β-endorphin and
    β-lipotropin in humans. Life Sci 29:1265-2169

Basbaum AI, Marley N, O'Keefe J, and Clanton CA 1977 Reversal of
    morphine and stimulus-produced analgesia by subtotal spinal
    cord lesions. Pain 3:43-56

Basbaum AI, and Fields HL 1978 Endogenous pain control mechanisms: review and hypothesis. Ann Neurol 4:451-462

Bhattacharya SK, Keshary PR, and Sanyal AK 1978 Immobilization stress-induced antinociception in rats: possible role of serotonin and prostaglandins. Eur J Pharmacol 50:83-85

Bjorklund A, Moore RY, Nobin A, and Stenevi U 1973 The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain. Brain Res 51:171-191

Blalock JE, and Smith EM 1981 Human leukocyte interferon (Hu IFN-a): Potent endorphin-like opioid activity. Biochem Biophys Res Commun 101:472-478

Bliss EL, Thatcher W, and Ailion J 1972 Relationship of stress to brain serotonin and and 5-hydroxyindoleacetic acid. J Psychiat Res 9:71-80

Bloch B, Bugnon C, Fellman D, and Lenys D 1978 Immunocytochemical evidence that the same neurons in the human infundibular nucleus are stained with antiendorphins and antisera of the other related peptides. Neurosci Lett 10:147-152

Bloom FE, Hoffer BJ, Siggins GR, Barker JL and Nicoll RA 1972 Effects of serotonin on central neurons. Microiontophoretic administration. Federation Proceedings 31:97-106

Bloom F, Battenberg E, Rossier J, Ling N, Leppaluoto J, Vargo TM, and Guillemin R 1977 Endorphins are located in the intermediate and anterior lobes of the pituitary gland not in the neurohypophysis. Life Sci 20:43-48

Bloom F, Battenberg E, Rossier J, Ling N, and Guillemin R 1978a Neurons containing beta-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. Proc Natl Acad Sci USA 75:1591-1595

Bloom FE, Rossier J, Battenberg ELF, Bayon A, French E, Henricksen SJ, Siggins GR, Segal D, Browne R, Ling N, and Guillemin R 1978b β-END: cellular localization, electrophysiological and behavioral effects In: Costa E, and Trabucchi M (eds) Advances in Biochemical Psychopharmacology: The Endorphins. Raven Press, New York

Bodnar RJ, Glusman M, Brutus M, Spiaggia A, and Kelly DD 1979 Analgesia induced by cold water stress: Attenuation following hypophysectomy. Physiol Behav 23:53-62

Bradbury AF, Feldberg WF, Smyth DG, and Snell C 1976 Lipotropin C-fragment: an endogenous peptide with potent analgesic activity. In: Kosterlitz HW, (ed) Opiates and Endogenous Opioid Peptides, North-Holland, Amsterdam

Briaud B, Koch B, Lutz-Bucher B, and Mialhe C 1979 In vitro regulation of ACTH release from neurointermediate lobe of rat hypophysis. Neuroendocrinology 28:377-385

Bruni JF, Hawkins RL, and Yen SSC 1982 Serotonergic mechanism in the control of β-endorphin and ACTH release in male rats. Life Sci 30:1247-1254

Buckingham JC, and Hodges JR 1979 Hypothalamic receptors involved in the secretion of corticotropin releasing factor. J Endocrinol 80:57P

Butcher LL, Engel J, and Fuxe K 1972 Behavioral, biochemical and histo-chemical analyses of the central effects of monoaminergic precursors after peripheral decarboxylase inhibition. Brain Res 41:387-411

Carr DB, Bergland R, Hamilton A, Blume H, Kasting N, Arnold M, Martin JB, and Rosenblatt M 1982 Endotoxin-stimulated opioid peptide secretion: Two secretory pools and feedback control in vivo. Science 217:845-848

Chang K-J, and Cuatrecasas P 1979 Multiple opiate receptors. J Biol Chem 254:2610-2618

Chang K-J, Cooper BR, Hazum E, and Cuatrecasas P 1979 Multiple opiate receptors: differential regional distribution in the brain and dif-ferential binding of opiates and opioid peptides. Mol Pharmacol 16:91-104

Chang K-J, Hazum E, and Cuatrecasas 1980 Multiple opiate receptors. TINS July 160-162

Chernick V 1981 Endorphins and ventilatory control. N Engl J Med 304:1227-1228

Chretien M, Benjannet S, Dragon N, Seidah NG, and Lis M 1976 Isola-tion of peptides with opiate activity from sheep and human pitui-taries: relationship to β-LPH. Biochem Biochem Biophys Res Commun 72:472-478

Cote TE, Eskay RL, Frey EA, Grewe CW, Munemura M, Stoof JC, Tsuruta K, and Kebabian JW 1982 Biochemical and physiological studies of the beta-adrenoceptor and the D-2 dopamine receptor in the inter-mediate lobe of the rat pituitary gland: A review. Neuroendo-crinology 35:217-224

Cox BM, Opheim KE, Teschemacher H, and Goldstein A 1975 A peptide-like substance from pituitary that acts like morphine. 2. Purifi-cation and properties. Life Sci 16:1777-1782

Cox BM, Goldstein A, and Li CH 1976 Opioid activity of a peptide, β-lipotropin-(61-91), derived from β-lipotropin. Proc Natl Acad Sci USA 73:1821-1823

Crine P, Gossarol F, Seidah NG, Blanchetti L, Lis M, and Chretien M 1979 Concomitant synthesis of β-END and α-MSH from two forms of pro-opiomelanocortin in the rat pars intermedia. Proc Natl Acad Sci USA 76:5085-5089

Csontos K, Rust M, Hollt V, Mahr W, Kromer W, and Teschemacher HJ 1979 Elevated plasma β-endorphin levels in pregnant women and their neonates. Life Sci 25:835-844

Curzon G, and Green AR 1970 Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. J Pharmacol 39:653-657

Cuthbert B, Holaday JW, Meyerhoff JL, and Li CH 1983 Cardiorespiratory and behavioral responses to intravenous β-endorphin in conscious rhesus monkeys. (in preparation).

Dahlstrom A, and Fuxe F 1964 Evidence for the existence of monoamine containing neurons in the CNS. I. Demonstration of monoamine cell bodies of brain stem neurons. Acta Physiol Scand 62, suppl. 232:1-55

Deakin JFW, Dostrovsky JO, and Smyth DG 1980 Influence of N-terminal acetylation and C-terminal proteolysis on the analgesic activity of β-END. J Biochem 189:501-506

Descarries L, and Beaudet A 1978 The serotonin innervation of adult rat hypothalamus In: Vincent J-D, and Kordon C (eds) Cell Biology of Hypothalamic Neurosecretion. Paris, pp 135-153

Dubois M, Pickar D, Cohen MR, Roth YF, Macnamara T, and Bunney Jr., WE, 1981 Surgical stress in humans is accompanied by an increase in plasma beta-endorphin immunoreactivity. Life Sci 29:1249-1254

Duncan C, and Spencer PSJ 1973 An interaction between morphine and fenfluramine in mice. J Pharm Pharmacol 25:124P-125P

Eipper B, and Mains R 1978 Existence of a common precursor to ACTH and endorphin in the anterior and intermediate lobes of the rat pituitary. J Supramol Struct 8:247-262

Eipper BA, and Mains RE 1980 Structure and biosynthesis of pro-adrenocorticotropin/endorphin and related peptides. Endocrine Rev 1:1-27

Eipper BA, and Mains RE 1981 Further analysis of post-translational processing of β-endorphin in rat intermediate pituitary. J Biol Chem 256:5689-5695

Farah JM Jr., Sapun-Malcolm D, and Mueller GP 1982 Dopaminergic inhibition of pituitary β-endorphin-like immunoreactivity secretion in the rat. Endocrinology 110:657-659

Farah JM Jr., Sapun-Malcolm D, and Mueller GP 1982 Dopaminergic mechanisms inhibit the release of immunoreactive beta-endorphin from

both the anterior and intermediate lobes of the rat pituitary. The Physiologist 25:261 (36.1)

Feldberg W, and Smyth DG 1976 Analgesia produced in cats by the C-fragment of lipotropin and by a synthetic pentapeptide. J Physiol 260:30P-31P

Fernstrom JD, and Wurtman RJ 1971 Brain serotonin content: Physiological dependence on plasma tryptophan levels. Science 173:149-152

Ferreira SH 1981 Inflammatory pain, prostaglandin hyperalgesia and the development of peripheral analgesics. TIPS July p. 183-186

Fields HL, Emson PC, Leigh BK, Gilbert RFT, and Iverson LL 1980 Multiple opiate receptor sites on primary afferent fibers. Nature 284: 351-355

Florez J, Mediavilla A, and Pazos A 1980 Respiratory effects of β-endorphin, D-Ala-met-enkephalinamide, and met-enkephalin injected in the lateral ventricle and the pontomedullary subarachinoid space. Brain Res 199:197-206

Fratta W, Yang T, Majane B, and Costa E 1979 Distribution of β-endorphin and related peptides in the hypothalamus and pituitary. Neuroscience 4:1903-1908

Fuller RW 1980 Pharmacology of central serotonin neurons. Annu Rev Pharmacol Toxicol 20:111-123

Fuller RW 1981 Serotonergic stimulation of pituitary-adrenocortical function in rats. Neuroendocrinology 32:118-127

Fuxe K, Schubert J, Hokfelt T, and Jonsson G 1974 Some aspects of the interrelationship between central 5-hydroxytryptamine neurons and hormones In: Advances in Biochemical Psychopharmacology: Serotonin vol 10, Raven Press, New York, pp. 67-74

Gallager DW, and Aghajanian GK 1976 Inhibition of firing of raphe neurones by tryptophan and 5-hydroxytryptophan: blockade by inhibiting serotonin synthesis with Ro-4-4602. Neuropharmacology 15:149-156

Ganong WF, Kramer N, Salmon J, Reid IA, Lovinger R, Scapagnini U, Boryczka AT, and Shackelford R 1976 Pharmacologic evidence for inhibition of ACTH secretion by a central adrenergic system in the dog. Neuroscience 1:167-174

Gautray J, Jolivet A, Vielh J, and Guillemin R 1977 Presence of immunoassayable β-endorphin in human amniotic fluid: Elevation in cases of fetal distress. Am J Obst Gynecol 129:211-212

Gilman SC, Schwartz JM, Milner RJ, Bloom FE, and Feldman JD 1982 β-Endorphin enhances lymphocyte proliferative responses. Proc Natl Acad Sci USA 79:4226-4230

Giraud P, Lissitzky J-C, Conte-Devolx B, Gillioz P, and Oliver C 1980 Influence of haloperidol on ACTH and β-endorphin secretion in the rat. Eur J Pharmacol 62:215-217

Gispen WH, Wiegant VM, Bradbury AF, Hulme EC, Smyth D, Snell CR, and de Wied D 1976 Induction of excessive grooming in the rat by fragments of lipotropin. Nature 264:794-795

Goldstein A, Lowney LI, and Pal BK 1971 Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of the mouse brain. Proc Natl Acad Sci USA 68:1742-1747

Goldstein A 1976 Opioid peptides (endorphins) in pituitary and brain. Science 193:1081-1806

Goldstein A, Tachibana S, Lowney LI, Hunkapiller M, and Hood L 1979 Dynorphin-(1-13), an extraordinarily potent opioid peptide. Proc Natl Acad Sci USA 76:6666-6670

Goodman RR, Snyder SH, Young WS, and Kuhar MJ 1980 Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. Proc Natl Acad Sci USA 77:6239-6243

Grahame-Smith DG 1971 Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J Neurochem 18:1053-1060

Grandison L, and Guidotti A 1977 Stimulation of food intake by muscimol and beta-endorphin. Neuropharmacology 16:533-536

Grau JW, Hyson RL, Maier SF, Madden J, and Barchas JD 1981 Long-term stress-induced analgesia and activation of the opiate system. Science 213:1409-1411

Greenwood FC, Hunter WM, and Glover JS 1963 The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. J Biochem 89:114-123

Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, and Blood F 1977 β-Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. Science 197:1367-1369

Harvey JA, Schlosberg AJ, and Yunger LM 1974 Effect of p-chlorophenylalanine and brain lesions on pain sensitivity and morphine analgesia in the rat In: Costa E, Gessa GL, and Sandler M (eds) Serotonin: New Vistas, Advanc Biochem Psychopharmacol vol 10, Raven Press, New York, pp 232-246

Hazum E, Chang KJ, and Cuatrecasas P 1979a Specific nonopiate receptors for β-endorphin. Science 205:1033-1035

Hazum E, Chang KJ, and Cuatrecasas P 1979b Interaction of iodinated human (D-ala)B-endorphin with opiate receptors. J Biol Chem 254: 1765-1767

Henderson G, Hughes J, and Kosterlitz HW 1972 A new example of a morphine-sensitive neuroeffector junction: adrenergic transmission in the mouse vas deferens. Br J Pharmacol 46:764-766

Herbert E, Roberts J, Phillips M, Allen R, Hinman M, Budarf M, Policastro P, and Rosa P 1980 Biosynthesis, processing and release of corticotropin, β-END, and MSH in pituitary cell cultures systems. In: Martini L, and Ganong WF (eds) Frontiers in Neuroendocrinology Vol 6 Raven Press, New York

Herkenham M, anad Pert CB 1980 In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiatergic" pathways. Proc Natl Acad Sci USA 77:5532-5536

Hiller JM, Pearson J, and Simon EJ 1973 Distribution of stereospecific binding of the potent narcotic analgesic etorphine in the human brain: Predominance in the limbic system. Res Commun Chem Pathol Pharmacol 6:1052-1062

Hiller JM, and Simon EJ 1979 ^{3}H-Ethyl-ketazocine binding: lack of evidence for a separate κ receptor in rat CNS. Eur J Pharmacol 60:389-390

Hokfelt T, Elde R, Johansson O, Terenius L, and Stein L 1977 The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. Neurosci Lett 5:25-31

Holaday JW, Loh HH, and Li CH 1978a Unique behavioral effects of β-endorphin and their relationship to thermoregulation and hypothalamic function. Life Sci 22:1525-1536

Holaday JW, Wei E, Loh HH, and Li CH 1978b Endorphins may function in heat adaption. Proc Natl Acad Sci USA 75:2923-2927

Holaday JW, and Loh HH 1979 Endorphin-opiate interactions with neuroendocrine systems In: Loh HH, and Ross DH (eds) Neurochemical Mechanisms of Opiates and Endorphins. Adv Biochem Psychopharmacol Vol 20, Roven Press, New York pp 227-258

Holaday JW (1983) Cardiovascular effects of endogenous opiate systems. Ann Rev Pharmacol Toxicol 23:541-594

Hollt V, Przewlocki, and Herz A 1978 Radioimmunoassay of β-endorphin basal and stimulated levels in extracted rat plasma. Naunyn-Schmiedeberg's Arch Pharmacol 303:171-174

Hosobuchi Y, Adams JE, and Linchitz R 1977 Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. Science 197:183-186

Hosobuchi Y, Rossier J, Bloom FE, and Guillemin R 1979 Stimulation of human periaqueductal gray for pain relief increases immunoreactive β-endorphin in ventricular fluid. Science 203:279-281

Houck JC, Kimball C, Chang C, Pedigo NW, and Yamamura HF 1980 Placental β-endorphin-like peptides. Science 207:78-80

Houghten RA, Swann RW, and Li CH 1980 β-Endorphin: Stability, clearance behavior, and entry into the central nervous system after intravenous injection of the tritiated peptide in rats and rabbits. Proc Natl Acad Sci USA 77:4588-4591

Howe A 1973 The mammalian pars intermedia: A review of its structure and function. J Endocrinol 59:385-409

Hughes J 1975 Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. Brain Res 88:295-308

Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, and Morris HR 1975 Identification of two related pentapeptides from the brain with potent opiate activity. Nature 258:577-579

Hughes J, Kosterlitz HW, and Smith TW 1977 The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. Br J Pharmacol 61:639-647

Hughes J 1981 Peripheral opiate receptor mechanisms. Trends Pharmacol Sci 2:21-24

Hylden JLK, and Wilcox GL 1983 Intrathecal serotonin in mice: analgesia and inhibition of a spinal action of substance P. Life Sci 33:789-795

Hyyppa MT, Cardinali DP, Baumgarten HG, and Wurtman RJ 1973 Rapid accumulation of H^3-tryptophan: Effects of 5,6-dihydroxytryptamine or female sex hormones. J Neural Transm 34:111-122

Jacobowitz DM, and O'Donohue TL 1978 α-MSH sitmulating hormone: immunohistochemical identification and mapping in neurons of rat brain. Proc Natl Acad Sci USA 75:6300-6304

Johns MA, Azmitia EC, and Krieger DT 1982 Specific in vitro uptake of serotonin by cells in the anterior pituitary of the rat. Endocrinology 110:754-760

Jones MT, Hillhouse EW, and Burden J 1976 Effect of various putative neurotransmitters on the secretion of corticotropin-releasing hormone from the rat hypothalamus in vitro - A model of the neurotransmitters involved. J Endocrinol 69:1-10

Kitahata LM, Kosaka Y, Taub A, Bonikos K, and Hoffert M 1974 Lamina-specific suppression of dorsal horn unit activity by morphine sulfate. Anesthesiology 41:39-48

Kosterlitz HW, Lord JAH, Paterson SJ, and Waterfield AA 1980 Effects of changes in the structure of enkephalins and of narcotic analgesic drugs on their interactions with μ- and δ-receptors. Br J Pharmacol 68:333-342

Krieger DT, Liotta A, and Brownstein MJ 1977 Presence of corticotropin in brain of normal and hypophysectomized rats. Proc Natl Acad Sci USA 74:648-652

Kuhar M, Pert C, and Snyder S 1973 Regional distribution of opiate receptor binding in monkey and human brain. Nature 245:447-450

Kulkarni SK 1980 Heat and other physiological stress-induced analgesia: Catecholamine-mediated and naloxone reversible response. Life Sci 27:185-188

Lang RE, Bruckner UB, Hermann K, Kempf B, Rascher W, Sturm V, Unger T, and Ganten D 1982 Effect of hemorrhagic shock on the concomitant release of endorphin and enkephalin-like peptides from the pituitary and adrenal gland in the dog. In: Costa E, and Trabucchi M (eds) Advances in Biochemical Psychopharmacology. Raven Press, New York pp 548-553

Law P-Y, Loh HH, and Li CH 1979 Properties and localization of β-endorphin receptor in rat brain. Proc Natl Acad Sci USA 76:5455-5459

Lewis JW, Cannon JT, and Liebeskind JC 1980 Opioid and nonopioid mechanisms of stress analgesia. Science 208:623-625

Lewis JW, Chundler EH, Cannon JT, and Liebeskind JC 1981 Hypophysectomy differentially affects morphine and stress analgesia. Proc West Pharmacol Soc 24:323-326

Lewis VA, and Gebhart GF 1977 Evaluation of the periaqueductal central gray (PAG) as a morphine-specific locus of action and examination of morphine-induced and stimulation-produced analgesia at coincident PAG loci. Brain Res 124:283-303

Li CH 1964 Lipotropin, a new active peptide from pituitary gland. Nature 201:924

Li CH, and Chung D 1976 Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. Proc Natl Acad Sci USA 73:1145-1158

Li CH, Chung D, and Doneen BA 1976 Isolation, characterization and opiate activity of β-endorphin from human pituitary glands. Biochem Biophys Res Commun 72:1542-1547

Liebeskind JS, Guilbaud G, Besson JM, and Oliveras JL 1973 Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effect on spinal cord interneurons. Brain Res 50:441-446

Lin MT, Chi ML, Chandra A, and Tsay BL 1980 Serotonergic mechanisms of beta-endorphin- and clonidine-induced analgesia in rats. Pharmacology 20:323-328

Liotta AS, Suda T, and Krieger DT 1978 β-LPH is the major opioid-like peptide of human pituitary and rat pars distalis: lack of significant β-END. Proc Natl Acad Sci USA 75:2950-2954

Liotta AS, Loudes C, McKelvy JF, and Krieger DT 1980 Biosynthesis of precursor corticotroin/endorphin-, corticotropin-, α-MSH, β-LPH-, and β-END-like material by cultured neonatal rat hypothalamic neurons. Proc Natl Acad Sci USA 77:1880-1884

Liotta AS, Yamaguchi H, and Krieger DT 1981 Biosynthesis and release of β-endorphin-, N-acetyl β-endorphin-, β-endorphin (1-27)-, and N-acetyl-β-endorphin (1-27)-like peptides by rat neurointermediate lobe: β-endorphin is not further processed by anterior lobe. Neurosci 1:585-595

Lissitsky JD, Morin O, Dupont A, Labrie F, Seidah NG, Cretien M, Lis M, and Coy DH 1978 Content of β-LPH and its fragments (including endorphins) in anterior and intermediate lobes of the bovine pituitary gland. Life Sci 22:1715

Lissitsky JC, Giraud P, Conte-Devolx B, Gillioz P, Boudouresque F, Eskay RL, and Oliver C 1980 β-Endorphin is present in high concentration in the hypophysial portal vessels of rats. Neurosci Lett 19:191-195

Loh HH, Tseng LF, Wei E, and Li CH 1976 β-Endorphin is a potent analgesic agent. Proc Natl Acad Sci USA 73:2895-2898

Lord JAH, Waterfield AA, Hughes J, and Kosterlitz HW 1976 Multiple opiate receptors In: Kosterlitz HW (ed) Opiates and Endogenous Opioid Peptides, Elsevier/North-Holland, Amsterdam pp 275-280

Lord JAH, Waterfield AA, Hughes J, and Kosterlitz HW 1977 Endogenous opioid peptides: multiple agonists and receptors. Nature (Lond) 267:495-500

Lowry PJ, Hope J, and Silman RE 1976 The evolution of corticotropin, melanotropin and lipotropin. Excerpta Med Int Congr Ser 402:71-76

Lytle LD, Messing RB, Fisher L, and Phebus L 1975 Effects of chronic corn consumption on brain serotonin and the response to electric shock. Science 190:692-694

Mains RE, and Eipper BA 1976 Biosynthesis of adrenocorticotropic hormone in mouse pituitary tumor cells. J Biol Chem 251:4115-4120

Mains RE, Eipper BA, and Ling N 1977 Common precursor to corticotropins and endorphins. Proc Natl Acad Sci USA 74:3014-3018

Mains RE, and Eipper BA 1978 Coordinate synthesis of corticotropins and endorphins by mouse pituitary tumor cells. J Biol Chem 253:651

Mains RE, and Eipper BA 1979 Synthesis and secretion of corticotropins, melanotropins, and endorphins by rat intermediate pituitary cells. J Biol Chem 254:7885-7894

Mains RE, and Eipper BA 1981 Differences in post-translational processing of β-endorphin in rat anterior and intermediate pituitary. J Biol Chem 256:5683-5688

Malick JB, and Goldstein JM 1976 Analgesia following intracerebral administration of enkephalin in the rat. The Pharmacologist 18:120

Martin WR, Eades GG, Thompson JA, Huppler RE, and Gilbert PE 1976 The effects of morphine and nalorphine-like drugs in the nondependent morphine-dependent chronic spinal dog. J Pharmacol Exp Ther 197: 518-532

Mayer DJ, Wolfe TL, Akil H, Carder B, and Liebeskind JC 1971 Analgesia from electrical stimulation in the brainstem of the rat. Science 174:1351-1354

Mayer DJ, and Hayes R 1975 Stimulation-produced analgesia: Development of tolerance and cross-tolerance to morphine. Science 188:941-943

Mayer DJ, and Price DD 1976 Central nervous system mechanisms of analgesia. Pain 2:379-404

Meisenberg G, and Simmons WH 1983 Peptides and the blood-brain barrier. Life Sci 32:2611-2623

Meites J, Bruni JF, Van Vugt DA, and Smith AF 1979 Relation of endogenous opioid peptides and morphine to neuroendocrine functions. Life Sci 24:1325-1336

Messing RB, Phebus L, Fisher L, and Lytle LD 1975 Analgesic effect of fluoxetine HCl (Lilly 110140), a specific uptake inhibitor for serotonergic neurons. Psychopharm Commun 1:511-521

Messing RB, Fisher L, Phebus L, and Lytle LD 1976 Interaction of diet and drugs in the regulation of brain 5-hydroxyindoles and the response to painful electric shock. Life Sci 18:707-714

Messing RB, and Lytle LD 1977 Serotonin-containing neurons: their possible role in pain and analgesia. Pain 4:1-21

Messing RB, Vasquez BJ, Spiehler VR, Martinez JL Jr, Jensen RA, Rigter H, and McGaugh JL 1980 ^{3}H-Dihydromorphine binding in brain regions of young and aged rats. Life Sci 26:921-927

Meyerson BJ, and Terenius L 1977 β-endorphin and male sexual behavior. Eur J Pharmacol 42:191-192

Mezey E, Leranth C, Friedman E, Krieger DT, Brownstein MJ, and Palkovitz M 1983 On the serotonin innervation of the rat pituitary inter-

mediate lobe. 13th Annual Society for Neuroscience Abstracts p 81 (25.9)

Mialhe C, and Briaud B 1977 Possible involvement of the pars intermedia of the rat pituitary in neurogenic stress. Front Hormone Res 4:193 199

Moon HD, Li CH, and Jennings BM 1973 Immunohistochemical and histochemical studies of pituitary β-lipotropins. Anat Res 175:524-538

Moore KE, and Johnston CA 1981 The median eminence: aminergic control systems. In: Muller EE, and MacLeod RM (eds) Neuroendocrine Perspective Vol 1, Elsevier Biomedical Press, pp 23-68

Moriarty GC, Halmi NS, and Moriarty CM 1975 The effect of stress on the cytology and immunocytochemistry of pars intermedia cells in the rat pituitary. Endocrinology 96:1426-1436

Morley JE 1980 The neuroendocrine control of appetite: the role of the endogenous opiates, cholecystokinin, TRH, gamma-amino-butyric-acid and the diazepam receptor. Life Sci 27:355-368

Moss IR, and Friedman E 1978 β-Endorphin: effects on respiratory regulation. Life Sci 23:1271-1276

Mueller GP, Twohy CP, Chen HT, Advis JP, and Meites J 1976 Effects of L-tryptophan and restraint stress on hypothalamic and brain serotonin turnover and pituitary TSH and prolactin release in rats. Life Sci 18:715

Mueller GP 1980 Attenuated pituitary beta-endorphin release in estrogen-treated rats. Proc Soc Exp Biol Med 165:75

Mueller GP 1981 Beta-endorphin immunoreactivity in rat plasma: variations in response to different physical stimuli. Life Sci 29: 1669-1674

Mueller GP, Pettibone DJ, Deeter WT, Sapun DI, and Farah JM Jr. 1981 In vivo release of beta-endorphin immunoreactivity by rat pars distalis: inhibition by glucocorticoids. 63th Annual Endocrine Society Abstracts p 182 (397)

Mueller GP, and Maiewski S 1983 Anesthesia and stimulation of pituitary beta-endorphin release. 65th Annual Endocrine Society Abstracts p 371(1162)

Muller EE, Nistico G, and Scapagnini U 1977 Neurotransmitters and Anterior Pituitary Function, Academic Press, New York

Nakanishi S, Inoue A, Kita T, Nakamura M, Chang ACY, Cohen SN, and Numa S 1979 Nucleotide sequence of cloned cDNA for bovine corticotropin-β-LPH precursor. Nature (London) 278:423-427

Nakao K, Nakai Y, Oki S, Matsubara S, Koniski T, Nishitani H, and Imura
H 1980 Immunoreactive β-endorphin in human cerebrospinal fluid.
J Clin Endocrinol Metab 50:230-233

Nemeroff CB, Osbahr III AJ, Manberg PJ, Ervin GN, and Prange Jr AJ
1979 Alterations in nociception and body temperature after intra-
cisternal administration of neurotensin, β-endorphin, other endo-
genous peptides and morphine. Proc Natl Acad Sci USA 76:5368-5371

Noble EP, Wurtman RJ, and Axelrod J 1967 A simple and rapid method for
injecting H^3-norepinephrine into the lateral ventricle of the rat
brain. Life Sci 6:281

Ng LKY, Chase TN, Colburn RW, and Kopin LJ 1972 Release of [^{3}H]dopa-
mine by L-5-hydroxytryptophan. Brain Res 45:499

O'Donohue TL, Miller RL, Pendleton RC, and Jacobowitz DM 1979 A diur-
nal rhythm of immunoreactive α-MSH in discrete regions of the rat
brain. Neuroendocrinology 29:281-287

Oleson TD, and Liebeskind JC 1975 Relationship of neural activity in
the raphe nuclei of the rat to brain stimulation-produced analgesia.
The Physiologist 18:338

Oliveras JL, Hosobuchi Y, Redjemi R et al. 1977 Opiate antagonist, nalo-
xone, strongly reduces analgesia induced by stimulation of a raphe
nucleus (centralis inferior). Brain Res 120:221-229

Olschowka JA, O'Donohue TL, Mueller GP, and Jacobowitz DM 1982 The dis-
tribution of corticotropin releasing factor-like immunoreactive
neurons in rat brain. Peptides 3:995-1015

Orwoll ES, and Kendall JW 1980 β-Endorphin and adrenocorticotropin
in extrapituitary sites: Gastrointestinal tract. Endocrinology
107:438-442

Oyama T, Jin T, Yamaya R, Ling N, and Guillemin R 1980 Profound anal-
gesic effects of β-endorphin in man. The Lancet, January 19,
p 122-124

Palkovits M, Brownstein M, Kizer JS, Saavedra JM, and Kopin IJ 1976
Effect of stress on serotonin concentration and tryptophan hydroxy-
lase activity of brain nuclei. Neuroendocrinology 22:298-304

Pardridge WM 1979 The role of blood-brain barrier transport of tryp-
tophan and other neutral amino acids in the regulation of substrate
limited pathways of brain amino acid metabolism. J Neural Trans,
Suppl 15:43-54

Pasternak GW, Goodman R, and Snyder SH 1975 An endogenous morphine-like
factor in mammalian brain. Life Sci 16:1765-1769

Pasternak GW, and Hahn EF   1980a   Long-acting opiate agonists and antag-
    onists: 14-hydroxydihydromorphinone hydrazones.   J Mednl Chem   23:
    674-676

Pasternak GW, Childers SR, and Snyder SH   1980b   Opiate analgesia: Evi-
    dence for mediation by a subpopulation of opiate receptors.   Sci-
    ence   208:514-516

Pasternak GW, Childers SR, and Snyder SH   1980c   Naloxozone, a long-act-
    ing opiate antagonist: Effects on opiate receptor binding and anal-
    gesia in intact animals.   J Pharmacol Exp Ther   214:455-462

Pelletier G, Leclerc R, Labrie F, Cote J, Chretien M, and Lis M   1977
    Immunohistochemical localization of β-lipotropic hormone in the
    pituitary gland.   Endocrinology   100:770-776

Pert C, and Snyder S   1973   Opiate receptor: Demonstration in nervous
    tissue.   Science   179:1011-1014

Peroutka SJ, Lebovitz RM, and Snyder SH   1979   Serotonin receptors
    affected differentially by guanine nucleotides.   Molec Pharmacol
    16:700-708

Peroutka SJ, and Snyder SH   1981   Two distinct serotonin receptors:
    regional variations in receptor binding in mammalian brain.   Brain
    Res   208:339-347

Peroutka SJ, Lebovitz RM, and Snyder SH   1981   Two distinct central sero-
    tonin receptors with different physiological functions.   Science
    212:827-829

Petraglia F, Penalva A, Genazzani AR, and Muller EE   1982   Stimulation
    of β-endorphin and β-lipotropin release from the anterior but not
    the neurointermediate pituitary lobe in the rat after acute admin-
    istration of serotonin-acting drugs.   Life Sci   31:2809-2817

Pettibone DJ, and Mueller GP   1982   Adrenergic control of immunoreactive
    β-endorphin release from the pituitary of the rat: In vitro and in
    vivo studies.   J Pharmacol Expt Ther   222:103-108

Ponzio F, Consolazione A, Calderini G, Achilli G, Rocchetti M and Algeri
    S   1981   Biochemical effects of quipazine on rat striatal dopaminer-
    gic system.   Life Sci   29:83-91

Przewlocki MJ, Millan MJ, Gramsch CH, Millan MH, and Herz A   1982   The
    influence of selective adeno- and neurointermedio-hypophysectomy
    upon plasma and brain levels of β-endorphin and their response to
    stress in rats.   Brain Res   242:107-117

Proudfit HK, and Anderson EG   1975   Morphine analgesia: blockade by raphe
    magnus lesions.   Brain Res   98:612-618

Proux-Ferland L, Labrie F, Dumont D, Cote J, Coy DH, and Sveiraf J 1982 Corticotropin-releasing factor stimulates secretion of melano-cyte-stimulating hormone from the rat pituitary. Science 217:62-63

Randic M, and Yu H 1976 Effects of 5-hydroxytryptamine and bradykinin in cat dorsal horn neurons activated by noxious stimuli. Brain Res 111:197-203

Rapoport SI, Klee WA, Pettigrew KD, and Ohno K 1980 Entry of opioid peptides into the central nervous system. Science 207:84-86

Reinhard JF Jr, Moskowitz MA, Sved AF, and Fernstrom JD 1980 A simple, sensitive and reliable assay for serotonin and 5-HIAA in brain tissue using liquid chromatography with electrochemical detection. Life Sci 27:905-911

Reynolds DV 1969 Surgery in the rat during electrical analgesia in-duced by focal brain stimulation. Science 164:444-445

Richardson DE, and Akil H 1977 Pain reduction by electrical stimu-lation in man Part 2. Chronic self-administration in the peri-ventricular gray matter. J Neurosurg 47:184-194

Rivier C, Brownstein M, Speiss J, Rivier J, and Vale W 1982 In vivo corticotropin-releasing factor-induced secretion of adrenocortico-tropin, β-endorphin, and corticosterone. Endocrinology 110:272-278

Rivier C, and Vale W 1983 Interaction of corticotropin-releasing factor and arginine vasopressin on adrenocorticotropin secretion in vivo. Endocrinology 113:939-942

Roberts JL, and Herbert E 1977 Characterization of a common precursor to corticotropin and β-lipotropin: Identification of β-lipotropin pep-tides and their arrangement relative to corticotropin in the pre-cursor synthesized in a cell-free system. Proc Natl Acad Sci USA 74:5162-5165

Roberts JL, Phillips M, Roase PA, and Herbert E 1978 Steps involved in the processing common precursor forms of adrenocorticotropin and endorphin in cultures of mouse pituitary cells. Biochemistry 17:3609-3618

Rossier J 1982 Opioid peptides have found their roots. Nature 298:221-222

Rubinstein M, Stein S, Gerber LD, and Udenfriend S 1977 Isolation and characterization of the opioid peptides from rat pituitary: β-LPH. Proc Natl Acad Sci USA 74:3052-3055

Samanin R, and Valzelli L 1971 Increase of morphine-induced analgesia by stimulation of the nucleus raphe dorsalis. Eur J Pharmacol 16:298-302

Samanin R, Ghezzi D, Mauron C, and Valzelli L 1973 Effect of midbrain raphe lesion on the antinociceptive action of morphine and other analgesics in rats. Psychopharmacologia (Berl) 33:365-368

Samanin R, Bernasconi S, and Quattrone A 1976 Antinociceptive action of quipazine: relation to central serotonergic receptor stimulation. Psychopharmacologia (Berl) 46:219-222

Schulz R, Faase E, Wuster M, and Herz A 1979 Selective responses for β-endorphin on the rat vas deferens. Life Sci 24:843-850

Sewell RDE, and Spencer PSJ 1974 Modification of the antinociceptive activity of narcotic agonists and antagonists by intraventricular injection of biogenic amines in mice. Br J Pharmacol 51:140P-141P

Sharpe LG, Garnett JE, and Cicero TJ 1974 Analgesia and hyperreactivity produced by intracranial microinjections of morphine into the periaqueductal gray matter of the rat. Behav Biol 11:303-313

Shields PJ, and Eccleston D 1973 Evidence for the synthesis and storage of 5-hydroxytryptamine in two separate pools in the brain. J Neurochem 20:881-888

Sicuteri F, Anselmi B, and DelBianco PL 1973 5-Hydroxytryptamine supersensitivity as a new theory of headache and central pain: a clinical pharmacological approach with p-chlorophenylalamine. Psychopharmacologia (Berl) 29:347-356

Simantov R, Kuhar MJ, Uhl GR, and Snyder SH 1977 Opioid peptide enkephaline: immunohistochemical mapping in rat central nervous system. Proc Natl Acad Sci USA 74:2167-2171

Simon EJ, Hiller JM, and Edelman I 1973 Stereospecific binding of the potent narcotic analgesic [^{3}H]etorphine to rat brain homogenate. Proc Natl Acad Sci USA 70:1947-1949

Sitsen JMA, Van Ree JM, and De Jong W 1982 Cardiovascular and respiratory effect of β-endorphin in anesthetized and conscious rats. J Cardiovasc Pharmacol 4:883-888

Smyth DG, Snell CR, and Massey DE 1978 Isolation of the C-fragment and C'-fragment from brain. J Biochem 175:261-270

Smyth DG, Massey DE, Zakarian S, and Finnie M 1979 Endorphins are stored in biologically active and inactive forms: isolation of α-N-acetyl peptides. Nature (London) 279(5710):252-254

Smyth DG, and Zakarian S 1980 Selective processing of β-endorphin in regions of porcine pituitary. Nature 288:613-615

Snyder S 1975 Opiate receptors in normal and drug altered brain function. Nature 257:185-189

Snyder SH, Childers SR, and Pasternak GW 1978 Opiate receptors: A) Functional heterogeneity demonstratead with an apparently irreversible naloxone derivative. B) regulation by guanine nucleotides In: Jacob J (ed) Advances in Pharmacology and Therapeutics Vol 1:Receptors Pergamon Press, New York pp 39-46

Snyder SH 1980 Brain peptides as neurotransmitters. Science 209:976-983

Snyder SH, and Goodman RR 1980 Multiple neurotransmitter receptors. J Neurochem 35:5-15

Telegdy G, and Vermes I 1973 The role of serotonin in the regulation of the hypophysio-adrenal system. In: Brodish A, and Redgate ES (eds) Brain-Pituitary-Adrenal Interrelationships. Karger, Basel pp 332-333

Tenen SS 1967 The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. Psychopharmacologia (Berl) 10:214-219

Tenen SS 1968 Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depletor. Psychopharmacologia (Berl) 12:278-285

Terenius L 1973 Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacol Toxicol 32:317-320

Terenius L, and Wahlstrom A 1975 Search for an endogenous ligand for the opiate receptor. Acta Physiol Scand 94:74-81

Teschemacher H, Opheim KE, Cox BM, and Goldstein A 1975 A peptide-like substance from pituitary that acts like morphine. I. Isolation. Life Sci 16:1771-1776

Thierry A-M, Fekete M, and Glowinski J 1968 Effects of stress on the metabolism of noradrenaline, dopamine and sertonin (5HT) in the central nervous system of the rat. (II) Modifications of serotonin metabolism. Eur J Pharmacol 4:384-389

Tilders F, and Smelik P 1977 Direct neural control of MSH secretion in mammals: the involvement of dopaminergic tubero-hypophyseal neurons In: Tilders F, Swaab D, and van Wimersma Greidanus Tj (eds) Frontiers of Hormone Research, Vol 4:80, Karger, Basel

Tilders FJG, Berkenbosch F, and Vermes I 1982 Control of β-endorphin secretion during emotional and systemic stress in rats and involvement of intermediate and anterior pituitary. 64th Endocrine Society Abstracts

Tozer TN, Neff NH, and Brodie BB 1966 Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. J Pharmacol Exp Ther 153:177

Trulson ME, and Trulson VM 1983 Chloral hydrate anesthesia alters the responsiveness of dorsal raphe neurons to psychoactive drugs. Life Sci 32:949-956

Tseng LF, Loh HH, and Li CH 1976 β-Endorphin as a potent analgesic by intravenous injection. Nature 263:239-240

Urca G, Frenk H, and Liebeskind JC 1978 Morphine and enkephalin: Analgesic and epileptic properties. Science 197:83-86

Usategui R, Oliver C, Vaudry H, Gaetano L, Rozenberg I, and Mourre AM 1976 Immunoreactive α-MSH and ACTH levels in rat plasma and pituitary. Endocrinology 98:189-196

Vale W, Rivier C, Yang L, Minick S, and Guillemin R 1978 Effects of purified hypothalamic corticotropin-releasing factor and other substances on the secretion of adrenocorticotropin and β-endorphin-like immunoreactivities in vitro. Endocrinology 103:1910-1915

Vale W, Rivier J, Guillemin R, and Rivier C 1979 Effects of purified CRF and other substances on the secretion of ACTH and β-endorphin-like immunoreactivities by cultured anterior or neurointermediate pituitary cells. In: Collu R, Barbeau A, Ducharme J, and Rocheforte JG (eds) Central Nervous System Effects of Hypothalamic Hormones and Other Peptides, Raven Press, New York p 163

Vale W, Vaughan J, Smith M, Yamamoto G, Rivier J, and Rivier C 1983 Effects of synthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neurohypophysial peptides, and other substances on cultured corticotropic cells. Endocrinology 113:1121-1131

Van De Kar LD, and Lorens SA 1979 Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei. Brain Res 162:45-54

van Leewen FW, Swaab DF, de Raay C, and Fisser B 1979 Immunoelectromicroscopical demonstration of α-MSH-like compound in the rat brain. J Endocrinol 80:59P-60P

Van Loon GR, and De Souza EB 1978 Effects of β-endorphin on brain serotonin metabolism. Life Sci 23:971-978

Van Loon GR, Ho D, and Kim C 1980 β-Endorphin-induced decrease in hypothalamic dopamine turnover. Endocrinology 106:76-80

Van Vugt DA, and Meites J 1980 Influence of endogenous opiates on anterior pituitary function. Fed Proc 39:2533-2538

Vermes I, Mulder GH, Smelik PG, and Tilders FJH 1980 Differential control of β-lipotropin secretion from anterior and intermediate lobes of the rat pituitary gland in vitro. Life Sci 27:1761-1768

Vincent SR, Hokfelt T, Christensson I, and Terenius L 1982 Dynorphin-immunoreactive neurons in the central nervous system of the rat. Neuroscience Lett 33:185-190

Vogt M 1974 The effect of lowering the 5-hydroxytryptamine content of the rat spinal cord on analgesia produced by morphine. J Physiol (Lond) 236:483-498

Vuolteenaho O, Vakkuri O, and Leppaluoto J 1980 Wide distribution of β-endorphin-like immunoreactivity in extrapituitary tissues of the rat. Life Sci 27:57-65

Waterfield AA, Leslie FM, Lord JAH, Ling N, and Kosterlitz HW 1979 Opioid activities of fragments of β-endorphin and of its leucine-analogue. Comparison of the binding properties of methionine- and leucine-enkephalin. Eur J Pharmacol 58:11-18

Watson SJ, Barchas JD, and Li CH 1977a β-LPH: Localization of cells and axons in rat brain by immunocytochemistry. Proc Natl Acad Sci USA 74:5155-5158

Watson SJ, Akil H, Sullivan SO, and Barchas JD 1977b Immunocytochemical localization of methionine-enkephalin: preliminary observations. Life Sci 25:733-738

Watson SJ, Akil H, Richard CW, and Barchas JD 1978 Evidence for two separate opiate peptide neuronal systems and the coexistence of β-lipotropin, β-endorphin, and ACTH immunoreactivities in the same hypothalamic neurons. Nature 275:226-228

Watson SJ, and Barchas JD 1979 Anatomy of the endogenous opioid peptides and related substances: The enkephalins, β-endorphin, β-lipotropin, and ACTH In: Beers RF, and Bassett EG (eds) Mechanisms of Pain and Analgesic Compounds, Raven Press, New York pp 227-237

Watson SJ, Akil H, and Barchas JD 1979 Immunohistochemical and biochemical studies of the enkephalins, β-endorphin, and related peptides. In: Usdin E, Bunney WE, and Kline NS (eds) Endorphins in Mental Health Research, Macmillan, New York

Watson SJ, and Akil H 1980a On the Multiplicity of active substances in single neurons: β-END and α-MSH as a model system In: de Wied D, and van Keep PA (eds) Hormones and the Brain, MTP Press, Lancaster, England

Watson SJ, and Akil H 1980b α-MSH in rat brain: Occurence within and outside brain β-END neurons. Brain Res 182:217-223

Watson SJ, Khachaturian H, Akil H, Coy DH, and Goldstein A 1982 Comparison of the distribution of dynorphin systems and enkephalin systems in brain. Science 218:1134-1136

Weber E, Voigt R, and Martin R 1978 Concomitant storage of ACTH and endorphin-like immunoreactivity in the secretory granule of anterior pituitary corticotrophs. Brain Res 157:385-390

Weiner RI, and Ganong WF 1978 Role of brain monoamines and histamine in regulation of anterior pituitary secretion. Physiol Rev 58:905-976

Westlund KN, and Childs GV 1982 Localization of serotonin and somatostatin fibers in the rat adenohypophysis. 12th Annual Society for Neuroscience Abstracts p 58 (18.31)

Winer BJ 1971 Statistical Principles in Experimental Design. McGraw-Hill, New York, p 907

Yaksh TL, DuChateau JC, and Rudy TA 1976 Antagonism by methysergide and cinanserin of the antinociceptive action of morphine administered into the periaqueductal gray. Brain Res 104:367-372

Zakarian S, and Smyth D 1979 Distribution of active and inactive forms of endorphins in rat pituitary and brain. Proc Natl Acad Sci USA 76:5972-5976